Meloidogyne, ALUMINUM, AND Rhizobium RELATIONSHIPS IN *Leucaena* GERMPLASM SELEC

BY

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by
Ann Rachel Hoffes

"From a single inflorescence
a thousand seedlings born
from this, the lifeline is
forever sown."

excerpted from
Oaxaca, 1982

This text is dedicated to
David
and to the memory of
Lou

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

Meloidogyne, ALUMINUM AND Rhizobium
RELATIONSHIPS IN Leucaene GERMPLASM SELECTION

BY

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Major Depertment: Agronomy

Interest in Leucaena spp. and its potential use as a forage and/or biomeee crop for Florida prompted e series of field, greenhouee, and leboretory studiee to screen the Leucaeua germplasm for several pest and soil related problems. Few diseaeas or pest problems heve been linked to Leucaena epp., but e low level of root-knot nemetode galling had been obeerved. Ninety-eight eccesions of Leucaena spp. were tested for resistauce to Florida populetione of two root-kuot nematodes, Meloidogyue lucoquite end M. javaulce. The results from the nematode etudy using three levels of nematode inoculum (0, 3, and 9 eggs cc ^{1} of soil), on 21-day old plants grown for an eddiitional 60 days in Conetainere, showed almost total resistance of the plant to either nematode species. A field study including 18 selected accessions grown in M. incognita infemted soil supported these findings.

[illegible] experiment using two Leucaena ecotypes (Br-[illegible] showed poor plant establishment and reduced yield[illegible] flatwoods soil plots (pH 4.21 in comparison t[illegible] activity on plots treated with $Ca(OH)_2$. A comparison was made of the growth of 58 Leucaena accessions screened in Conetainers® on three Florida soils, Bladen (pH 4.7), Wakka (pH 4.1), and Rockdale (pH 7.6). Significant differences in shoot height, plant dry weight, and nodulation of the accessions were observed after 75 days. Plant growth, in general, was poor for all accessions tested, when compared to their growth on the limed or near neutral soils. Accessions which may have tolerance to acid soils include ecotypes UF-1 and UF-2, PIs 262405, 299044, and 200295, and CIAT lines 78[illegible], 22/9-7, 17-5/2-27, and 16/1-7. The accessions also differed in response to acidity and/or Al in solution studies containing 5.0 ppm Ca with varying levels of Al (0.0, 0.5, 1.0, 2.0, and 4.0 ppm Al). Overall plant growth in solutions containing Al was poor.

Two acid tolerant strains of Rhizobium spp. which infect and fix nitrogen with Leucaena spp. were isolated from native legumes, and partially characterized. Isolates SS-10 and SS-46 grew well on acidified yeast mannitol agar plates and broth, pH 4.0, and 4.5, respectively. Bacterial count after 14 days of growth in the acid broth containing 50 ppm Al was 1×10^{-7} cells mL^{-1}.

CHA[illegible]
INTRODU[illegible]

[illegible] he most important tasks facing mankind are meeting world food and fuel requirements in an ever increasing demand. Renewable sources of food and energy play important roles for present and future agricultural production. A major problem is the low level of supply of dietary protein. One solution to augment food protein intake is to incorporate legumes into the diet. Exploitation of legumes to date has been limited to peanut, soybean, a few edible beans and peas, and a few forage legume species. The family Leguminosae offers a wealth of untapped potential, with approximately 750 genera and over 16,000 estimated species (Allen and Allen, 1981). Legumes present a means of reducing protein malnutrition, common particularly in developing countries, as well as contributing lumber, fuelwood, dyes and copal, fertilizer, forage, and a variety of other resources.

Among the legume genera, tropical species have been the least exploited, particularly those with potential forage and fuel value. Leucaena, with its 10 diverse species, has been described as one of the most promising of the legumes (Hutton, 1970; NAS, 1977).

[illegible] of small woody leguminous plants [illegible], show considerable promise as multipurpose crops for tropical and subtropical regions. Potential uses of Leucaena include timber and paper pulp, animal protein supplementation, green manure, biomass, and soil conservation. This diversity of uses for Leucaena and its ability to grow on marginal soils, with minimal input, have encouraged much recent international attention.

In Florida, forage legume crops are urgently needed to improve the protein status of perennial grass pastures. Legumes such as Leucaena, tolerant of the low fertility and water deficient soils, could contribute significantly to pasture improvement. Leucaena also offers a possible protein supplement for Florida's growing poultry and swine industries which currently depend heavily on imported supplemental protein feeds. The palatability and nutritive value of Leucaena leaves are comparable to alfalfa (NAS, 1977), and it provides a rich source of beta-carotene, desirable in poultry rations. The utilization of Leucaena as a quality feed supplement is overshadowed by the presence of an antiquality component, mimosine; however, selection for low-mimosine lines may overcome this limitation (Brewbaker and Hylin, 1965).

Leucaena also offers promise for soil reclamation, plantings on phosphate mine spoils, and use as a renewable biomass resource in the state. Limited knowledge of the

adaptability of the plant to Florida has supported the evaluation of the Leucaena germplasm at several research locations throughout Florida (Soto, 1982; Valencia, 1981; and Kretschmer, personal communication). Freezing winter temperatures in the northern part of the state generally cause some plant die-back. Poorly drained acid soils, prevalent throughout Florida, may also limit the productivity of the plant. Little information is available on the resistance of Leucaena to disease and insect problems which may further restrict its cultivation.

This study was designed to evaluate a portion of the Leucaena germplasm for tolerance to soil acidity, the effect of soil acidity on the Rhizobium symbiosis, and susceptibility of the germplasm to two prevalent nematode species (Meloidogyne incognita and M. javanica) in the state.

Agronomics of Leucaena Species

Botanical Classification

The genus Leucaena Bentham belongs to the family Mimosaceae of the order Leguminales [R. Br. in Flinders, Voy Terra Austr. 2:551 (1814)] as cited by Brewbaker (1978). Within the genus Leucaena, some 51 species have been recognized; however only 10 are considered to be taxonomically valid (Brewbaker, 1978). Among the 10 species, descriptions indicate considerable genetic variability within the germplasm. Most species are largely self-fertilized, yet natural hybridization does occur (Brewbaker and Hylin, 1965; Brewbaker et al., 1972; Gray, 1968).

Taxonomic studies by Brewbaker (1978) and Brewbaker and Ito (1980) divide the genus Leucaena into species based upon morphological and biochemical differences. Leucaena esculenta, L. pulverulenta, and L. trichodes are small-leafed (3 to 6 mm) plants with numerous leaflet pairs (20 to 60) having a chromosome number of 2n=52. Leucaena collinsii, L. lencocephala, and L. shannonl have an intermediate leaf size (6 to 24 mm) and fewer leaflet pairs

(19 to 35) Leucaena leucocephala thought to be an allotetraploid hybrid of L. collinsii and L. diversifolia (Brewbaker et al., 1972), has 2n-104 chromosomes. Leucaena lanceolata, L. macrophylla, L. retusa, and L. trichodes all have large leaflets (20-6 cm) and the fewest leaflets per pinna (4 to 7). Chromosome numbers of 2n-52 have been reported for L. lanceolata and L. trichodes.

Genetic variation within L. leucocephala is mostly quantitative, including photoperiod response, plant morphology, branching habit, pubescence, mimosine content, as well as other characteristics (Brewbaker et al., 1972). The species was grouped into three types by Hutton and Gray (1959) to distinguish among the major morphological differences. Type 1, the "Hawaiian" type, is bushy, short, non-photoperiod responsive, resulting in year round flowering. The plants are generally low-yielding, weedy types, found throughout the tropics. Type 2, the "Salvador" type, is tall and strongly arboreal, with little side-branching, producing more than twice the biomass of the Hawaiian types (NAS, 1977). These plants are late flowering and produce large pods and seeds, i. e., "Hawaiian Giants." The genetic base of the Salvador types is believed to be narrow, with most lines originating in Morazan, a province of Salvador (Brewbaker, 1980). These plants are excellent wood producers. Type 3, the "Peru" type is semi-arboreal, and was originally introduced from Peru (Brewbaker, 1980).

These [illegible] to be st[illegible]ly branched at the base, produc[illegible] [illegible]rass yields, suggesting excellent forage potenti[illegible]

Botanical [illegible]iption

Leucaena species as described by de Wit (1961) and Brewbaker (1978), are mostly small arboreal types or shrubs with great variation in branching habit (Hutton and Gray, 1959, Gray, 1960). The trunk is usually smooth and thin-barked, freckled or stippled and light-toned; red-brown or gray. Branching habit varies with species and type, but branches generally are smooth and void of thorns. As with branching, leaf variation is great. Leaves are bipinnate, with the number and size of pinnae and leaflets varying among the different species (Brewbaker, 1978). Glands, generally found between the basal and terminal pinnae pairs, vary in shape and number and have aided in the taxonomic definition of species. The flowers, consisting of many individual florets, occur as dense globular heads that are typically white, but may be pink or yellow at anthesis. Heads occur on short axillary peduncles. The flower is five parted and sessile, bearing 10 free stamens and a thin, long style. In L. leucocephala, anthesis occurs in the early morning with pollen falling directly onto the stigmas, resulting in a high degree of self pollination (Gray, 1968). In general, flowers are slightly dichogamous, with

pistils maturing 1 to 2 days aft[illegible] (Brewbaker, 1978; Gonzalez et al., 1967). Ait[illegible] florets average 1[illegible] per head, approximately 3 to 5% actually set seed (Gonzalez et al., 1967; Mamiopic and Empig, 1980). The young pods are usually green or red and mature to a dark brown. Seed color, shape, and size vary with species, but the seeds are typically ovate, flat, and brown. The leucaena plant has a deep taproot with few superficial or lateral roots.

Symbiosis with Rhizobium Species

Effective symbiosis is usually apparent on the root with large, multilobed nodules located predominantly along the central taproot (Allen and Allen, 1981). Nodules are considered to be indeterminate, having persistent meristems which are of graded age from the meristematic tip to the root attachment (Bergersen, 1982). The vascular system is typically open in this type of nodule anatomy with infection threads as the major mechanism for Rhizobium distribution within the nodule cells. Perennial nodules were not observed in Leucaena (Allen and Allen, 1981). Cross-inoculation studies in Brazil with root nodule bacteria indicated that L. leucocephala was highly strain-specific (Galli, 1958). Rhizobium isolates from Leucaena nodules were similar culturally and physiologically to the slow-growing strains generally infective on cowpea, lupine, and soybean (Allen and Allen, 1981). Trinick (1965) found

strain [illegible] morphology and carbohydrate utilization to be similar [illegible]ar of fast-growing Rhizobium spp. Norris (1965) found fast growing, acid-producing rhizobia were infective on Leucaena spp and he suggested that perhaps the bacteria were evolutionarily specialized, having a narrow symbiotic range, with special requirements for soil nutrient status and pH. Contrary to this, Wilson (1939) and Allen and Allen (1981) reported that nodulation on Leucaena spp is not as highly specialized as claimed. Leucaena glauca was nodulated by strains isolated from Desmanthus virgatus, Mimosa pudica, Prosopis juliflora and two Sesbania species (Allen and Allen, 1981). Nodulation by a Rhizobium strain isolated from a Cassia spp. was also noted on a Leucaena species grown in Hawaii (J. Halliday, personal communication). Wilson's studies in the 1930s suggest that the degree of selfing or outcrossing influences the tendency of that legume to nodulate with a number of different strains. Wilson grouped L. glauca (leucocephala) as progressing from self-pollinating towards cross-pollinating, nodulating with Rhizobium strains from species of nine different genera. Based on varying reports and observations, Trinick (1968) drew two basic conclusions: (1) L. leucocephala is nodulated solely by fast-growing, tropical Rhizobium strains, and (2) fast-growing Rhizobium strains of L. leucocephala can nodulate tropical legumes normally nodulated by slow-growing strains.

Uses and Potential

Leucaena species have great potential [illegible] feed and fuel problems in the tropics. In addition to forage, timber and firewood, other uses of Leucaena include organic fertilizer, shade, windbreaks, and soil conservation (NAS, 1977; Oakes, 1968). The plant exhibits rapid growth and an extensive deep tap-root system that allows for drought tolerance and growth on infertile soils. Major reviews on the genus Leucaena by Gray (1968), Oakes (1968), the National Academy of Science (1977), and Blom (1980) provide for more detailed information.

Environmental Considerations

Environmental factors which may limit the utilization of Leucaena include; rainfall shortage (Oakes and Butcher, 1962) and high elevations (Oakes, 1968; Blom, 1980). Growth potential may also be limited by temperature (Jones, 1979; Davies, 1960), soil factors (Blom, 1980; Oakes and Butcher, 1962; Dijkman, 1950), moisture (Dijkman, 1950, Takahashi, 1952), and shade (Dijkman, 1950, Oakes and Butcher, 1962).

It is well documented that Leucaena grows poorly on acid soils. Although not specific in its pH requirements (Dijkman, 1950), Leucaena appears well adapted to neutral or alkaline soils, while exhibiting poor growth on more acid soils (Hutton, 1979; Jones, 1979). Screening and breeding for acid soil tolerance in the germplasm have been ongoing

research locations around the world. Del [redacted] and Salapare (1980) reported that attempts at [redacted]ing Leucaena accessions for acid soil tolerance showed that primary root growth was pH sensitive and in some accessions, root growth was totally inhibited. Among Leucaena accessions tested, K8 appeared to be most inhibited, while k28 seemed unaffected. Hutton (1980, 1981, 1982) at CIAT, Colombia, selected Leucaena accessions with some degree of tolerance to highly acid soils and ultisols (pH 4.0 to 4.5) having high Al saturation (70 to 90%). Fertile hybrids from L. leucocephala x L. pulverulenta, backcrossed several times to L. leucocephala (cv. Cunningham) offered some degree of tolerance when grown in a soil mixture with a pH 4.5 and 90% Al saturation. Progenies of these crosses ranged from 5 cm in height, non-inoculated and chlorotic, to 20 cm, nodulated, green-leaved and thick-stemmed (Hutton, 1980). Further results from Hutton (1981) showed that little acid tolerance existed in L. leucocephala accessions, with plants exhibiting retarded growth and degrees of leaf yellowing. Variation for acid tolerance was found within lines of L. pulverulenta, while F_1 hybrids of L. leucocephala x L. pulverulenta which were backcrossed once to cv. Cunningham, with the following generations open-pollinated, were strongly acid tolerant. Relatively high percentages of plants with high acid tolerance were found among L. diversifolia (2 lines), L. macrophylla, and

L. shannonii accessions. Acid tolerance, deep root penetration of the soil and strong lateral root development appeared to be correlated (Hutton, 1981).

Genetics and Breeding of Leucaena for Acid Soil Environments

Plant breeding programs with Leucaena species have been concentrated mostly on agronomic improvement, such as decreasing mimosine content of the foliage and increasing plant biomass. Inter- and intra-strain variability have been observed (Hutton and Gray, 1959) and efforts have been made to transfer desirable traits into potential agronomic types.

Adapting Leucaena to acid soils, particularly oxisols and ultisols, involves consideration of both plant and Rhizobium. The well-weathered oxisols and ultisols typically have low base status (pH 5 or lower) and unavailable or deficient amounts of Ca, Mg, P, and K (Lotz et al., 1972). Aluminum, H, and Mn ions may be present in the soil solution at levels toxic to a growing plant (Kamprath and Foy, 1972). Depth of the Al saturation varies with the soil type (Hutton, 1982). Aluminum saturation of 85% or higher is not uncommon in the soils of the Colombian Llanos, and tolerant legume species must survive with 5 ppm Al in the soil (Salinas, 1980). Calcium may also become a limiting factor in these soils, and plant selection must also be based on efficient root absorption of Ca and deep root penetration. Variation in

[illegible]olerance and efficient Ca absorption has been noted [illegible] the Leucaena species (Hutton, 1982). Hutton found [illegible]r analysis differences in "giant" L. leucocephala 78-[illegible]o be due to poor Ca absorption rather than lack of Al [illegible]ance. Leucaena diversifolia and natural hybrids of L. macrophylla and L. shannoni had both tolerance to Al [illegible]d good Ca absorption, resulting in vigorous growth and deep penetration of roots into the soil. Olvera et al. (1982) suggested establishing plants in small pots containing limed, fertilized soil. Pots, with bottoms removed, were then placed in acid soils. Results on the sandy spodosols (pH 4.6) where tested appeared beneficial for initial root establishment; however, this technique did not insure root growth and normal development of the plants.

[illegible]d Tolerance in the Rhizobium Symbiosis

The growth and infectivity of Rhizobium at a low soil pH must also be considered for successful growth of Leucaena on acid soils. Infection and nodulation of the host plant appears to be somewhat pH dependent (Munns, 1976; Date and Norris, 1979). Acid and Al tolerant Rhizobium may not be a limiting factor, however, since such strains have been isolated and grown on an acidified yeast extract mannitol media (Young and Chao, 1982) and an arabinose sodium glutamate medium (Aquilahuatl and Munoz, 1983). Norris (1972) reported that strain CB 89 was more

[illegible] L. leucocephele. Strain 1145, isolated and [illegible] Hawaii, is also considered highly acid [illegible] capable of infecting Leucaena species under acid soil conditions (Helliday, 1981).

Insects and Diseases

Leucaena species have long been described as virtually disease and insect free (NAS, 1977); however, due to its now pantropical distribution, many observations on plant pathogenicity have been reported. Oakes (1968) reviewed the literature up to that time on disease and insect occurrence on Leucaena. Recent reports have identified a range of tropical plant-pathogen and insect problems, including; Fusarium sp. (Haleh, 1982, Lenne and de Alverez, 1982, Perez Guerrero, 1982), Pseudomonas flourescens Biotype 2 (Lenne and Torres, 1982), Rhizoctonie solani (Perez Guerrero, 1982), Cercospora sp. (Perez-Guerrero, 1982), Colletotricum sp. (Hsieh, 1982, Perez-Guerrero, 1982), Camptomeris leucaenae (Haleh, 1982; Lenne, 1980), gummosis disease (Dutt, 1982, Patil et al., 1982), Pythium spp. Hsieh, 1982), and insect problems associated with scale insects (Cheng et al., 1982), seed weevils, twig borers, and termites (NAS, 1977).

Nematological information concerning Leucaena is rather scarce although references to root-knot nematode,

[illegible]idogyne spp., infection on Leucaena spp. has been [illegible]d in two articles (Massey, 1911; Thrower, 1958). [illegible]re infection of L. glauca roots by M. javanica was [illegible]d in Papau, New Guinea (Thrower, 1958). Nematode-[illegible]ted plants were killed outright or severely galled [illegible]duced yield compared with the uninoculated control [illegible], mature females and egg masses were recovered, [illegible]n that the nematode successfully reproduced in [illegible] plan tissue. More recently, resistance to M. [illegible]ncognita in L. leucocephala was reported by Hasan et al. (1983). Differences in the degree of nematode pathogenicity may be due, in part, to biotypes of the nematode, and environmental and host differences.

Soil Factors Affecting Plant Growth and Development

Soil pH affects nutrient absorption and plant growth directly, by the effect of the H ion, and indirectly, due to the influence on toxic ions and nutrient availability. Brady (1974) and Tisdale and Nelson (1975) describe these effects in great detail. In general, plant availability of K, Ca, Mo, and Mg tend to increase as the soil pH increases, while Fe, Mn, and Zn tend to become less available as the pH increases from 5.0 to 8.0. Phosphorus is most available to the plant at a pH around 6.5. At pH values less than 5.0, however, Al, Fe, and Mn concentrations in the soil may be toxic to the growth of certain plants (Brady, 1974; Tisdale and Nelson, 1975).

Several reviews discuss the effects of pH on soil plant growth (Foy, 1974; Munns, 1976, Reid, Foy, Sartain, 1974) and the reader is directed to those for further information.

Aluminum injury is often expressed when the soil pH falls below about 5.2 where Al is free in the soil solution. Raising the pH above 5.2 to 5.5 generally precipitates Al and prevents plant injury (Reid, 1976). Crop injury due to Al in the soil first appears in the roots, with reduced root elongation, thickening and abnormal branching, and necrosis or discoloration of the root tip (Fleming and Foy, 1968; Reid, 1976). The mechanisms involved in Al damage include interference with normal uptake and utilization of P (Clarkson, 1966, Foy and Brown, 1963, Ragland and Coleman, 1962; Robinson, 1965; Wright and Donahue, 1953), interference with Ca uptake (Clarkson and Sanderson, 1971; Jackson, 1967; Johnson and Jackson, 1964; Lund, 1970), inhibition of cell division (Clarkson, 1965; Sampson et al., 1965) and restriction of the permeability of plant roots (Clarkson and Sanderson, 1971; Drew and Biddulph, 1969; Lance and Pearson, 1969).

Aluminum Effect on Phosphorus Uptake and Utilization

Much research has revealed that Al affects P uptake and utilization in the plant. Studies with labeled P^{32} and Al with barley showed Al accumulation on the root surface and in the cortex, while little P translocation to the tops of the plants occurred (Wright and Donahue, 1953). Phosphorus is thought to be precipitated in solution by Al to form aluminum phosphate (Ragland and Coleman, 1962, Rorison, 1965). Clarkson (1966) proposed that two interactions between P and Al occur: (1) fixation and precipitation of P occurs on the surface or in the free space between plant root cells, making P less available for metabolic activities, and (2) when Al penetrates the plant cell, it interferes with cell division, sugar phosphorylation, and DNA synthesis (Clarkson, 1966, Clarkson and Sanderson, 1971, Rorison, 1965, Sampson et al., 1965).

Aluminum Effect on Calcium Uptake and Transport

The effect of Al on Ca uptake is well documented (Foy, 1974, Johnson and Jackson, 1964; Clarkson and Sanderson, 1971, Lance and Pearson, 1969). Johnson and Jackson (1964) found that Al inhibited uptake of Ca by the plant. They suggested the relationship between the two ions is one of competition for exchange or carrier sites. If the inhibiting effect of Al on Ca is solely competition, then

[illegible] ons in solution [illegible] diffe[illegible] uptake by the plant.

Various [illegible] Al levels to better [illegible] interac[illegible] two cations has given dittering res[illegible] Sanderson (1971) found that inhibi[illegible]e in barley was overcome when Ca l[illegible] in the growth medium while Al leve s remained constant. Lund (1970) raported slmllar flndings with soybean, where Al toxicity was overcome by increasing Ca from 10 to 40 ppm in solutions oontalnlng 1 ppm Al. Conflicting results were reported by Johnson and Jackson (1964) when varylng the Ca concentration wlth two levels of Al showed a parallel relationship. In addltion to this, while the rate of Ca uptake did not appear to be affected by Al levels, the actual amount of Ca taken up by the plant was reduced. Further studies lndicated Al effects on the metabolic activity of the cell mlght lndlrectly lnfluence the uptake of Ca. Drew and Biddulph (1969) showed that Ca appears not to be actlvely transported across the plasmalemma. Clarkson and Sanderson (1971) suggested that the effect of Al is non-specific for Ca, but rather is an electrical affinity, whereby the trivalent Al ions compete with and displace divalent Ca ions on the root surface.

Aluminum Effect on Cell Division and Elongation

Plant cell elongation and cell division have long been thought to be sensitive to Al concentrations in the soil solution. Clarkson (1965) found Al to inhibit all elongation in onion roots at micromolar concentrations. Fleming and Foy (1968) noticed that Al sensitive soybean plants exhibited disorganization of the root cap, vascular elements, and root apex. The Al insensitive cultivar appeared to have a gelatinous matrix surrounding its root cap. Aluminum appeared to be acting on the DNA of newly elongating and dividing cells. When Al stress was removed, damaged rootlets did not recover, but lateral and adventitious root growth was initiated.

Differential Responses of Plant Species and Cultivars to Aluminum

Species and cultivars within species generally differ in response to Al. Both multiple and single gene resistances have been reported and tolerance has been transferred within species using conventional breeding procedures. Lutz and coworkers (1971) found tolerance to Al in corn grown on an acid soil (pH of 3.9) to be a dominant trait. Gorsline and associates (1964) reported response of corn to Al appeared to be heritable and possibly under the control of three major genes. In studies with rice,

[illegible] tolerated Al-
tolerance in a several high y[illegible] cultivars. Reid (1976)
reported that tolerance to Al was due to a single dominant
[illegible] in barley, as did Kerridge and Kronstad (1968) from
their work with wheat varieties. Other investigations
suggest that more than one major gene is involved in Al
tolerance in plants. Foy and coworkers (1965) found a wide
range in Al response in 27 wheat varieties, suggesting Al
tolerance in wheat is not simply inherited.

Fioldy level may also influence Al tolerance in plants.
In wheat, hexaploid varieties appeared to have the highest
tolerance to soil acidity among Triticum spp. (Slootmaker,
1974). Further reference to this phenomenon is found in a
review on Al toxicity by Reid (1976).

Low soil pH appears to favor grasses over legumes,
perhaps due to the effects of low pH on the Rhizobium
symbiosis (Spencer, 1950; Lonergan, 1960). Nevertheless,
legume species do differ in their adaptation to a wide range
of soil environments. Several legume species found to be
relatively insensitive to up to 75 µM Al include Desmodium
uncinatum, Macroptilium lathyroides, Trifolium
ruseppellianum, and Lotononis bainesii (Andrew et al., 1973).
Among the temperate legumes, Trifolium repens, T.
semipilosum (Andrew et al., 1973), and T. subterraneum
(Munns, 1965b) are more intermediate in their acid

tolerance [illegible] ast, Medicago sativa and M. scutellata were more sensitive to soil Al levels and growth at 20 µM Al in solution ([illegible] in soil) was retarded. Growth was totally inhibited at 75 d0 µM Al (Andrews et al., 1973; Munns, 1965a). Considerable variation in cultivar response of M. sativa to Al, however, has been recently reported by Brooks et al. (1982).

Aluminum Screening of Plant Species--Methodology

Foy (1976) has comprehensively reviewed screening plants for tolerance to Al. In general, plant screening requires reliable, rapid techniques for large-scale germplasm evaluation. In selecting a soil medium, interactions of Al with other mineral elements present, soil pH, texture, cation exchange capacity, and other chemical and physical properties of the medium must be considered. Screening plants in nutrient solutions also has its limitations, since constant monitoring of salts, P, Ca, and solution pH is necessary.

Several indices have been used to compare plant tolerance to Al. Shoot dry weight (Armiger et al., 1968), root dry weight (McLean and Gilbert, 1927), and number of roots (Kerridge et al., 1971) all appear to correlate well with plant tolerance to Al.

Root structure also serves as a good indicator of differential Al tolerance in plants. Fleming and Foy (1968)

found disorganization of the root apex, root cap, and vascular tissues of cv. Monon wheat roots occurred when the plant roots were exposed to Al, compared with an Al-tolerant (Atlas 66) variety. Atlas 66 exhibited a protective gelatinous matrix covering the root. The Al sensitive cultivar resumed normal root growth of the lateral roots once the roots were removed from the Al solution.

Root cation exchange capacity has also been used to predict Al tolerance in plants. Huffaker and Wallace (1958) found low cation exchange capacity of the roots tends to favor uptake of monovalent over divalent or trivalent (i.e., Al) cations. A similar relationship was reported in wheat roots by Foy et al. (1967).

Nutrient solution screening of plants is one method of evaluating the degree of plant tolerance to Al. Sartain (1974) compared soybean varietal growth in solutions containing varying levels of Al and 10 ppm Ca at a pH of 4.6. Initial root length, final root length, and a post treatment root measurement were taken to evaluate root growth and root regrowth potential during and after the Al stress exposure. Differences in root length were corrected for by using the RER (mean relative extension rate) value (May et al., 1965) which accounts for initial root length variation due to varietal differences. He found distinct differences in the degree of Al tolerance among the soybean varieties tested.

Al screening in nutrient solution[illegible]
[illegible] s by Sartain (1974), Foy (1976), [illegible]
[illegible]

Factors Affecting the Rhizobium Symbiosis

Soil pH Effects

Some strains of Rhizobium do grow and nodulate with their legume host at low soil pH, suggesting that tolerance to acidity varies among Rhizobium strains (Date and Halliday, 1979; Davina, 1976) as well as with the plant species (Damirgi et al., 1967; Munns, 1976; Norris, 1965). While some strains of Rhizobium do not grow even at moderate acidities, other strains survive and replicate successfully at low pH (Alexander, 1982). Munns (1968a) suggested that an acid sensitive step in the nodulation process exists in some legume-Rhizobium relationships that results in the inhibition or repression of the symbiosis. Differences in acid response are often great, as with R. japonicum, where serogroups vary as much as three units in their minimum pH tolerance for growth (Damirgi et al., 1967). Similar findings were reported among 50 Rhizobium strains isolated from Stylosanthes spp. and grown on standard yeast mannitol (YM) medium at pH 6.8 to 7.0 and pH 4.5 (Date and Halliday, 1979). Some strains were found to grow much better at the more acid pH level. In general, critical low pH levels for

Rhizobium [illegible]th range from about 4.0 to [illegible] meliloti considered acid sensitive and R. japonicum, R. lupini, and the cowpea miscellany considered to be more more tolerant (Keyser and Munns, 1979b).

Aluminum Effects

Heavy metal toxicity of Al (Keyser and Munns, 1979a; Carvalho et al., 1981), Mn (Reisenaur et al., 1962; Doberreiner, 1966), or inadequate levels of Ca, P, and Mo (Graham and Hubbell, 1973) in acid soils may affect the nutrient status of the legume, indirectly affecting the Rhizobium symbiosis. Keyser and Munns (1979a) reported growth differences existed among cowpea Rhizobium strains grown in medium containing varying concentrations of Al (25-100 μM). Aluminum was found to delay nodulation in several species of Stylosanthes, and to reduce the number and dry weight of nodules in all six species tested (Carvalho et al., 1981).

Calcium Effects

Although the Ca requirements for Rhizobium in pure culture are low (Vincent, 1962), Rerkasem (Ph.D. thesis cited by Keyser and Munns, 1979b) found Ca prevented the decline of viability of a fast growing Rhizobium strain in an acid medium. Slow growing cowpea miscellany strains were found to be more acid tolerant and did not respond to Ca

[illegible] and Munns (1979a) supported this, finding [illegible] effect of Ca on Rhizobium growth with varying [illegible] in liquid culture.

In early [illegible] Lipkin and Sommer (1928, 1940), Ca deficiency appeared to disrupt normal mitosis and subsequent cell division. Li[illegible] (1974) suggested that Ca may be necessary for normal cell division involved in root-nodule development. Further information on the effect of soil factors affecting the legume-Rhizobium symbiosis can be found in reviews by Munns (1976), Lie (1974), and Graham and Hubbell (1973).

Aluminum Screening of Rhizobium-Methodology

Numerous methods have been cited in the literature as means to evaluate the effects of Al on Rhizobium growth and replication. In particular, two in vitro methods appear feasible for large-scale assessment of Rhizobium strains. Keyser and Munns (1979b) evaluated 65 Rhizobium strains by means of visually detectable turbidity in nutrient broth media with low pH and P, and high Al. Assuming that detectable turbidity was indicative of Rhizobium growth, they found significant differences among the strains tested. A second method of agar plate-screening Rhizobium cultures for acid and Al tolerance was reported by Ayanaba et al. (1983). Date and Halliday (1979) used an acid agar plate procedure for selecting acid tolerant Rhizobium. They

Rhizobium

of tolerance to Al among those

d on colony growth and morphology.

Although Rhizobium strains do vary in their ability to tolerate Al in culture and soil, Alexander (1982) suggests that research emphasis should be placed more on plant response to Al than on the Rhizobium tolerance.

CHAPTER III
[illegible] OF Leucaena SPECIES GERMPLASM FOR GENETIC [illegible] TO Meloidogyne incognita AND M. javenice PATHOGENICITY

Introduction

Nematode susceptibility of cultivated crops is of widespread importance. Two species of root-knot nematode [Meloidogyne incognita (Kofoid and White) Chitwood and M. javenica (Treub) Chitwood] are prevalent in warmer soils, and their pathogenicity affects a wide range of crops. The current international interest in Leucaena spp. suggested a need to evaluate the response of this tree legume to root-knot nematodes. A low incidence of root-knot nematode galling was observed at Gainesville, Florida, during some preliminary trials with a forage type Leucaena. Conflicting reports of root-knot nematode pathogenicity on Leucaena are cited in the literature (Hessey, 1911; Thrower, 1958). This study was initiated to evaluate a part of the USDA Leucaena plant introduction collection for response to local Florida races of M. incognita and M. javanica.

Materials and Methods

Experiment 1

Ninety-seven Leucaena accessions (Table 3-1) and Florida common alyceclover, Alysicarpus vaginalis (L.) DC.,

[illegible] List of Alysicarpus and Leucaena accessions used in experiments 1 and 2.

[illegible]	Species	[illegible]
[illegible]	A. vaginalis	Florida
[illegible]	L. leucocephala	Florida
[illegible]	L. leucocephala	Florida
[illegible]	L. leucocephala	U.S. [illegible] Islands
[illegible]	L. leucocephala	U.S. [illegible] Islands
[illegible]	L. leucocephala	Brazil
[illegible]	L. leucocephala	Philippines
[illegible]	L. leucocephala	Belgian Congo
[illegible]	L. esculenta	Mexico
[illegible]	L. leucocephala	South Africa
[illegible]	L. leucocephala	India
[illegible]	L. leucocephala	Taiwan
[illegible]	L. leucocephala	U.S. Virgin Islands
[illegible]	L. leucocephala	Colombia
[illegible]	L. leucocephala	Australia
[illegible]	L. leucocephala	Tanganyika
[illegible]	L. leucocephala x L. pulverulenta	New Guinea
[illegible]	L. leucocephala x L. glabrata	New Guinea
28[illegible]	L. leucocephala	New Guinea
281[illegible]	L. leucocephala	New Guinea
281[illegible]	L. leucocephala	New Guinea
281777	L. leucocephala	New Caledonia
28177[illegible]	L. leucocephala	New Caledonia
281779	L. leucocephala	New Caledonia
281780	L. leucocephala	Puerto Rico, U.S.A
281781	L. leucocephala	Ceylon
28178[illegible]	L. leucocephala	Ghana
281783	L. leucocephala	Sierra Leone
281784	L. leucocephala	Senegal
282340	L. leucocephala	Philippines
282404	L. leucocephala	Colombia
28240[illegible]	L. sp.	Honduras
282458	L. leucocephala	Colombia
282461	L. insularum	Australia
282462	L. leucocephala	Australia
28246[illegible]	L. leucocephala	Australia
282464	L. leucocephala	Australia
282465	L. leucocephala	Australia
28246[illegible]	L. leucocephala	Australia

[illegible] nued.

28[illegible]	L. laucocephala	Au[illegible]
282 [illegible]	L. laucocaphala	Aus[illegible]
282[illegible]	L. laucocaphala	Australia
282[illegible]	L. laucocaphala	Australia
282471	L. laucocaphala	Australia
282470	L. laucocaphala	Australia
282473	L. laucocaphala	Australia
282474	L. sp.	Australia
282692	L. leucocaphala	Mexico
282817	L. leucocaphala	Taiwan
283697	L. leucocaphala	New Caledonia
286248	L. lancaolata	Mexico
286295	L. leucocephala	Ivory Coast
288001	L. laucocaphala	U.S.A.
288003	L. laucocaphala	U.S.A.
288004	L. laucocaphala	U.S.A.
288005	L. leucocephala	U.S.A.
288007	L. laucocaphala	U.S.A.
288008	L. laucocaphala	U.S.A.
288009	L. pulverulenta	U.S.A.
288011	L. leucocephala	U.S.A.
292345	L. laucocaphala	Bolivia
295360	L. laucocaphala	Taiwan
295362	L. laucocephala	Taiwan
295363	L. leucocephala	Taiwan
295364	L. laucocaphala	Taiwan
300010	L. laucocaphala	South Africa
300011	L. laucocaphala	South Africa
304650	L. laucocaphala	Argentina
305453	L. leucocaphala	Sierra Leone
308544	L. leucocaphala	Colombia
308568	L. laucocephala	Venezuala
311128	L. laucocephala	Nicaragua
311513	L. laucocaphala	Brazil
317908	L. laucocaphala	U.S.A.
317909	L. laucocephala	U.S.A.
317910	L. laucocaphala	U.S.A.
317911	L. laucocephala	U.S.A.
317912	L. laucocephala	U.S.A.
317916	L. pulverulenta x L. laucocaphala	U.S.A.
317918	L. sp.	U.S.A.
322553	L. leucocaphala	Brazil
331738	L. laucocaphala	Australia
364373	L. laucocaphala	Nigeria
367835	L. leucocaphala	Argentina

[illegible]	[illegible]	U.S.A.
38[illegible]	[illegible]	Bahamas
3843[illegible]	[illegible]	Bahamas
4157[illegible]	[illegible]	Taiwan
4359[illegible]	[illegible]	U.S.A.
4434[illegible]	[illegible]ccephala	El Salvador
443[illegible]	[illegible]ocephala	Mexico
44350	[illegible]ocephala	Mexico
[illegible]	[illegible]ocephala	Mexico
[illegible]	[illegible]ocephala	Mexico
[illegible]	leucocephala	Mexico

ere germinated under sterile conditions (seeds were surface-sterilized in 95% EtOH for 30 sec, immersed in 0.1% acidified $HgCl_2$ for 3 min and rinsed six times in sterile, distilled, deionized water). Two day old seedlings were transplanted into 150 cc Conetainers® (Ray Leach Conetainer Nursery, Canby, Oregon) containing a steam-sterilized 1:1 mixture of Myakka fine sand (sandy, siliceous, hyperthermic Aeric Haplaquod) and coarse sand. The soil mixture was fertilized with a commercial micronutrient mixture (FTE 503) prior to autoclaving. Plants were watered once a week, with full strength Hoagland's solution (Hoagland and Arnon, 1938) or tap water as needed. At 21 days, seedlings were infested with 0, 3, or 9 eggs oc^{-1} soil of M. incognita supplied in a concentrated 1 mL aliquot applied with an automatic pipet to a depth of approximately 6 cm. Nematode inoculum was obtained from Dr. R. A. Dunn, Department of Entomology and Nematology, University of Florida. Conetainers® were arranged in a randomized complete-block design with five replications. Seedlings were grown under 30% shade using a shade screen. The seedlings were evaluated 60 days after inoculation and roots were scored on a visual rating scale of 0 to 5 (0 = no galls, 1 = 1 or 2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = > 100 galls per root system).

Experiment 2

This experiment was conducted at the same time and under the same conditions as Experiment 1, except that M. javanica eggs were substituted as the inoculum.

Experiment 3

Surface sterilized seeds (as described in Experiment 1) of 17 Leucaena accessions and Florida common A. vaginalis sp. (Table 3-2) were germinated aseptically. Two day-old seedlings were planted in Todd® Planter Flats (Speedling Manufacturing, Inc., Sun City, Florida) containing steam-sterilized commercial potting soil. At 30 days, half the seedlings were infested with approximately 9 eggs cc^{-1} soil of M. incognita eggs directly into the Todd® Planter Flats while the other half received no eggs and served as control plants. The infested and noninfested plants with attached root balls were then transplanted to a fumigated field treated with 37 L ha^{-1} of ethylene dibromide (Soilbrom-90 EC®, Great Lakes Chemical Corp.). The field was located on Myakka fine sand at the Beef Research Unit (BRU), University of Florida, Gainesville, Florida. The experimental design was a split-plot with nematode infestation versus no infestations as the main plots, and accessions as the subplots. There were eight replications. Plants were dug at 120 days of growth, and root gall counts (based on a 0 to 5 gall rating scale as described in Experiment 1) and whole plant dry matter yields were recorded.

List of Alysicarpus and Leucaena accessions used in Experiment 3.

Accession	Species	Origin
Alyceclover	A. vaginalia	Florida
UF-2	L. leucocephala	Florida
281771	L. leucocephala	New Guinea
281784	L. leucocephala	Senegal
282405	L. sp.	Honduras
282458	L. leucocephala	Colombia
282460	L. insularum	Australia
282465	L. leucocephala	Australia
282468	L. leucocephala	Australia
282471	L. leucocephala	Australia
282474	L. sp.	Australia
282692	L. leucocephala	Mexico
286009	L. pulverulenta	U.S.A.
295364	L. leucocephala	Taiwan
304650	L. leucocephala	Argentina
317918	L. sp.	U.S.A.
345516	L. leucocephala	Bahamas
443599	L. leucocephala	Mexico

[illegible] the data was performed using the [illegible] Sis System (SAS) and computing facili[illegible] Regional Data Center (NERDC) at the [illegible] Florida. Mean responses in Experiments 1, [illegible] pared using Duncan's Multiple Range Test at [illegible] nt dry matter yields in Experiment 3 were [illegible] rly among the Leucaena accessions tested. The [illegible] us dry matter yield was deleted from the [illegible] al analysis, although it is reported in the result[illegible] and discussion section.

Results and Discussion

Experiments 1 and 2

Assessment of root-knot nematode tolerance in Leucaena spp. and A. vaginalis grown in Conetainers® provided a relatively rapid and inexpensive method for screening large quantities of plants in a limited space. All Leucaena accessions tested showed only a low incidence of root galling in response to either M. incognita or M. javanica. Although gall ratings were statistically significant at the 0.05 level of probability, gall numbers were so low that biologically they appeared of little importance. Leucaena PI 286295 had a 1.6 gall rating at the 9 egg cc^{-1} inoculation level but no more than 2 or 3 galls per root system were observed. No apparent galling at the 3 egg cc^{-1} soil inoculum level was noted on PI 286295. One other

genotype, a hybrid of L. [illegible]ocephala / L. pulverulenta, PI 281767, had a gall score of 1 at the 3 egg cc^{-1} inoculum level, but only a 0.7 at the 9 eggs cc^{-1} level. All other accessions were rated to have gall scores of less than 1, and most accessions exhibited no galling at all.

The Florida common alyce clover accession, a highly root knot susceptible genotype, provided an excellent example of an intolerant plant response to both nematode species. Analysis of the data showed the Alysicarpus accession to have significantly greater galling at both nematode inoculum levels, compared to all Leucaena accessions in Experiments 1 and 2, and to the uninfested Alysicarpus plants. In several of the replications galling was so great that partial root decomposition actually reduced the root galling score.

Experiment 3

Field results in Experiment 3 correlated well with the shade house results obtained from Experiment 1. Nematode-infected Leucaena accessions did not show any root gall infection, compared to the infested A. vaginalis accession which was heavily galled (Table 3-3). In one replication, an uninoculated A. vaginalis entry showed appreciable galling (rated at 3), although planted in a fumigated soil. This is possibly due to resurgence of the nematode after fumigation, faulty fumigation, or root contamination from

[illegible] although not [illegible] usually higher for the [illegible] compared with the nematode [illegible] Alysicarpus [illegible] and the reverse was true for [illegible] grasses. Statistically significant dry matter [illegible] were measured among the Leucaena [illegible] (Table 3-3), however, the fumigation treatment had no significant effect on dry matter yield within the same accession. Among the accessions, UF-2 and PI 282405 exhibited early seedling vigor compared with the other Leucaena accessions, although the magnitude of the yield differences was small.

Summary and Conclusions

In efforts to make a general statement as to the high degree of root-knot nematode resistance in Leucaena spp., one must assume that all populations of M. javanica and M. incognita are the same. Races or biotypes do exist among Meloidogyne spp. These data suggest that Leucaena spp. do exhibit a high degree of tolerance to the races (biotypes) of M. incognita and M. javanica used in these experiments.

The highly root-knot susceptible Alysicarpus accession provided an excellent positive check which proved the viability of nematode inocula. Although 3 eggs cc^{-1} soil rates were suitable for an inoculum load in Conetainers®, instituting a "light" and a "heavy" inoculum load level as

[illegible]. [illegible] [illegible] incognita infes[illegible] and no[illegible]n [illegible] [illegible]na and Alysicarpus accessions for [illegible] response and dry matter yield [illegible] conditions.

[illegible]	Root gall rating† noninfest.	Root gall rating† infest.	Plant dry matter yield noninfest.	Plant dry matter yield infest.
	no.	no.	g plants^{-1}	g plants^{-1}
[illegible]	0.4 a‡	5.0 a	3530	2813
[illegible]	0.0 b	0.0 b	320 a*	325 a
[illegible]	0.0 b	0.0 b	89 bc	111 b
[illegible]	0.0 b	0.0 b	83 bc	124 b
282405	0.0 b	0.0 b	244 ab	230 ab
282458	0.0 b	0.0 b	131 bc	144 b
282460	0.0 b	0.0 b	224 abc	154 b
282465	0.0 b	0.0 b	154 bc	136 b
282468	0.0 b	0.0 b	203 abc	135 b
282471	0.0 b	0.0 b	146 bc	149 b
282474	0.0 b	0.0 b	93 bc	88 b
282692	0.0 b	0.0 b	111 bc	201 ab
288009	0.0 b	0.0 b	119 bc	86 b
295364	0.0 b	0.0 b	106 bc	70 b
304650	0.0 b	0.0 b	70 c	144 b
317918	0.0 b	0.0 b	91 bc	137 b
384516	0.0 b	0.0 b	79 c	127 b
443599	0.0 b	0.0 b	83 bc	143 b

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†Gall rating based on 0 to 5 scale as explained in the Materials and Methods section.

‡Means in the same column not followed by the same letter are significantly different at the 0.05 level.

*No significant overall treatment or treatment x genotype interaction affect.

[illegible] as an [illegible] check insured against gall [illegible]
[illegible] due to [illegible]ng" by the nematode. Root pr[illegible]
[illegible] to a phenomenon whereby the lateral roots have
[illegible]ly been pruned by the nematode and no apparent gal[illegible]
is observed; hence low gall scores would be recorded when
the plant actually has a marked response to the nematode.

CHAPTER
ACTERIZATION
Rhizobium SPECIES
Leucaena SPECIES

Introduction

Leucaena has a successful
with Rhizobium species. Although previous
shown that L. leucocephala grown on
at the Beef Research Unit (BRU) near
Florida (pH 4.0 4.5) were chlorotic, stu
lated, a few indigenous Rhizobium strains
effectively nodulated two L. leucocephala
is location. The objectives of the
ing investigations were 1) to serologically
Rhizobium strains isolated from L. leucocephala
acid soils, 2) to investigate the specificity of
tion of these strains with several native and
troduced legumes, and 3) to characterize the growth of
Rhizobium strains on artificial medium as affected by
, aluminum (Al), or phosphorus (P) level.

Materials and Methods

Experiment 1 Serological Identification of Strains

Approximately 80 isolations were made from L. leucocephala nodules of Rhizobium inoculated and

uninoculated plots grown on [illegible] soils at the BRU in [illegible]. Inoculated plots received a mixed peat based culture of TAL 1145, [illegible], and TAL 82 Rhizobium strains supplied by Dr. J. Halliday, NifTAL, Hawaii. Isolates were compared using the indirect fluorescent antibody (FA) technique (Schenk et al., 1978). Antiserum for Rhizobium strains A 309, TAL 1145, TAL 582, and TAL 82 were supplied by Dr. Halliday and Sheila May. Titer was determined using the FA system and the appropriate rabbit anti-bacteria immunoglobulin (Ig) diluted to the next to the lowest dilution that gave an observable reduction in fluorescence with homologous cells. Isolates were streaked and dried onto glass slides. Specific rabbit anti-sera diluted to 1:30 with a phosphate buffered saline (FTA, Hemagglutination Buffer®, BBL Division of Becton, Dickinson and Co.), pH 7.2, was applied to the preparations and incubated at 37°C for 25 min. The preparations were rinsed three times in FTA buffer prior to applying anti-rabbit fluorescein-conjugated goat Ig (1:20 dilution with FTA buffer). Slides were incubated again at 37°C for 25 min and rinsed three times in FTA buffer. Slides were mounted using buffered glycerol. Observations were made with a Leitz fluorescence microscope equipped with epi-illumination. Illumination was provided by a 100 watt high pressure mercury lamp. Visual ratings of the bacteria were scored on a 0 = none, 1 = dim, 2 = medium, and 3 = very bright

fluorescing reaction. Control slides included a non-related Rhizobium and TAL 1145, TAL 582, and TAL 82 Rhizobium cells.

Experiment 2--Preliminary Noduiation Studies

Rhizobium strains were isolated from root nodules of Cassia nictitans L., Galactia regularis (L.) BSP., Indigofera hirsuta L., Sesbania vesicaria (Jacq.) Ell., Tephrosia hispiduia (Michx.) Pers., Aeschynomene americana L., Crotalaria purshii D.C., and an introduced Leucaena leucocephala (Lam) de Wit. Verification of plant species was made by Dr. David Hall, Extension Botanist, Plant Identification and Information Service, Univ. of Florida. Nodules were surface sterilized with $HgCl_2$ as outlined by Vincent (1970), and plated on a modified (pH 6.0) yeast mannitol (YM) medium (Vincent, 1970). Rhizobium strains were purified by repeated isolation from single colonies (Table 4-1).

Growth pouch bioassay

A test for cross-inoculation of the native legume Rhizobium strains with Leucaena leucocephala was carried out using seedlings growing in disPo Seed-Pack growth pouches® (Scientific Products) as shown in Fig. 4-1. The 10 strain treatments were as follows: a control (no inoculation), a mixed culture of TAL 82, 582, and 1145; a mixed culture of SS-10, SS-39, and SS-46, A-101; BP-102, C-103, G-104; I-105;

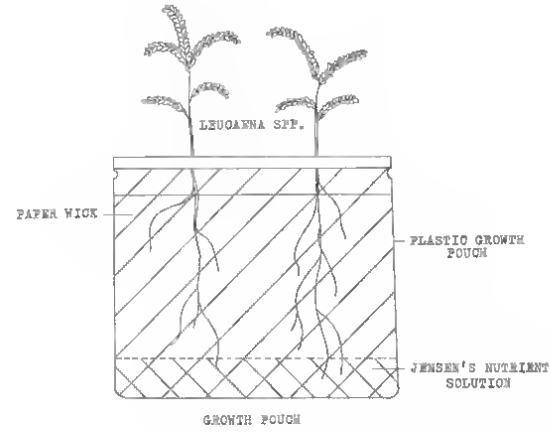

Fig. 4-1. Growth couch assay.

Two Leucaena accessions (UF 1 and UF
s ere scarified with emory cloth, surface-
0.2% acidified $HgCl_2$ (Vincent, 1970), and
.6°C in the dark in eterile Petri piates
were approximately 2 cm loug, one seedling
and UF-2 were piaced aseptically into gro th
po contained 25 mL of sterile Jensen's nutrient
incent, 1970). One milliliter aliguots of ths
Rhizobium cultures, grown for 24 hrs at 28°C in
sterile yeast mannitol broth, were asmptically
into each growth pouch. Each Rhizobium
ecding combination wes replicated eight times.
er placed under a fluorescent plant growth light
imately 25°C for 42 days, after which they ware
d for the presence of effectlve nodulae.

Gibson tube bioassay

A second method to avaiuate cross inoculation of native legume Rhizobium strains on Leucaena leucocephala wae conducted using plants growing in modified Gibson assemblies (Vincent, 1970) as shown in Fig. 4 2. The test tube size used was 25 mm x 175 mm. The nutrient solutiou used was that of Jansen (Vincent, 1970) with 15 g L^{-1} ager for the solid portion. Seeds of UF-1 and UF-2 were scarified and germinated as described previouely for the growth pouch bioassay, and single seedlinge were aseptically placed in the Gibson assemblies.

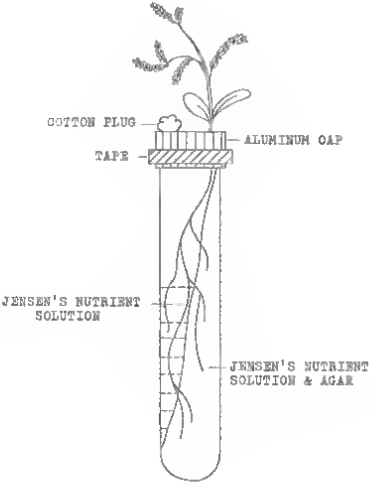

Fig. 4-2: Gibson tube assembly

[illegible] with Rhizobium studies [illegible] as described for the growth pouch [illegible], [illegible] 1 mL aliquots of the Rhizobium suspension were [illegible] into the nutrient solution. Sterile water was [illegible] in vial tubes as needed by the seedlings.

[illegible] treatment was repeated five times for the UF-1 and [illegible] ions. Plants were grown under fluorescent plant [illegible] lights at approximately 25°C for 60 days, and then [illegible] for presence of effective nodules.

Experiment 3 -Acid and Aluminum Tolerance Studies

Acid-triplate study

Concurrent with growth pouch and Gibson assemblies, all individual strains used in these studies were plated onto triplates containing yeast mannitol agar (Vincent, 1970) of varying pH (4.0, 6.0, and 8.0). The pH was adjusted with 1 N HCl or 1 N NaOH just prior to autoclaving. The agar media were poured into disposable Y-plate Petri dishes (100 by 15 mm) at 7 mL section^{-1} and allowed to solidify at 25°C for 3 hrs before inoculation. Inoculations were made using 0.4 µl from a suspension of 10^6 to 10^7 rhizobia mL^{-1} dilution of Rhizobium. Plates were incubated at 25°C and examined every third day for growth over an 18-day period. Colony growth was evaluated using a 0 to 5 scale where 0 = no growth to 5 = large gummy colonies.

Table 1. Strains of Rhizobium spp. used in nodulation studies.

Strain(s)	Isolated from	Origin
TAL 82, 582, 1145	Leucaena spp.	NifTAL, Hawaii
SS 10, 39, 46	Leucaena spp.	Gainesville, Florida
I-101	Aeschynomene americana	Gainesville, Florida
I-102	Cassia nictitans	Gainesville, Florida
I-103	Crotalaria purshii	Gainesville, Florida
G 104	Galactia regularis	Gainesville, Florida
I-105	Indigofera hirsuta	Gainesville, Florida
S 106	Sesbania vesicaria	Gainesville, Florida
I-107	Tephrosia hispidula	Gainesville, Florida

[redacted]

[redacted] ins of Rhizobium were selected for this study [redacted] effects of high levels of Al and low levels [redacted] on growth and survival at low pH, as well as [redacted] between acid-tolerance and Al tolerance among [redacted]. Three Rhizobium isolates (SS-10, SS-39, and [redacted] were isolatsd from nodules on Leucaena spp. grown at [redacted] ville, Florida. The fourth strain, TAL 1145, [redacted] rom NifTAL, Hawaii.

[redacted] studying the saprophytic growth of the Rhizobium, [redacted] lo sidearm flasks (Bellco Glass Co.) were usad. [redacted] Rhizobium was assessed by the decrease in [redacted] ttance at 580 nm with a spectrophotometer [redacted] ronic 20). Data were converted and presented [redacted] section as optical density (O.D.).

[redacted] ks were inoculated with an initial density of 10^2 to 10^{-1} [redacted] ml^{-1} media. Initial and final cell populations [redacted] ere estimated using a Petroff-Hauser counting chamber. Flasks were incubated in a constant temperature shaker at [redacted] 25 C and read daily. A final population count and pH [redacted] determination was made after 14 days.

The liquid media are described in Table 4-2. These media are modifications of original solutions described by [redacted] ke ser and Munns (1979b).

Acid media were acidified with 1 N HCl prior to [redacted] autoclaving. Aluminum was added after autoclaving as 50 µM $AlK(SO_4)_2$ solution sterilized by filtration.

Table 4-2. Media for acid broth cultures

Basal solution: mannitol 10 g L^{-1}; salts (μM) 300 $MgSO_4$, 300 $CaCl_2$, 10 ferric EDTA, 10 KCl, 1 $MnCl_2$, 0.4 $ZnSO_4$, 0.1 $CuCl_2$, 0.02 Na_2MoO_4, 0.002 $Co(NO_3)_2$, distilled water

Treatment	1	2	3	4	5	6
	0.5 g yeast	→	0.55 Na glutamate	→	→	
	500 μM KH_2PO_4	→	→	5 μM KH_2PO_4	500 μM KH_2PO_4	
	500 μM K_2HPO_4	→	→	1500 μM KCl	500 μM K_2HPO_4	50 μM $AlK(SO_4)_2$
pH adjusted	6.3	4.5	6.3	6.3	4.5	4.5

Experiment [illegible] Serological Identification of [illegible]

All [illegible] for non-specific binding and autoagglutination of isolates were negative. In general, nodule isolates [illegible] reacted predominantly with antiserum of TAL 1145; however, inconsistencies with the expression of flocculation among the tested antisera were observed (Table 4-3). This suggests that the field isolates, particularly those from non-inoculated plots, may be serologically related to TAL 1145, with similar surface or flagella antigens, but that they are different from any of the applied strains with the possible exception of strains L-82-8 and L-82-9 which fluoresced with the antiserum of TAL 1145. Of the strains isolated from inoculated plants, only strain 101-4 had a homologous reaction similar to the applied inoculum strain TAL 582, and similarly, strain L-83-9 with TAL 1145. These results suggest that native legume Rhizobium strains growing in this acid flatwoods soil are capable of competing with seed applied Rhizobium strains and nodulating L. leucocephala.

Experiment 2--Preliminary Noduiation Studies

The growth pouch and Gibson assemblies were effective means for determining whether Rhizobium strains from native legumes would nodulate Leucaena spp. Strains that were

Table 4-3. Characterization and cross-reactivity of selected Rhizobium field isolates.

Bacterial strain	Plot treatment	Antisera tested			
		TAL 1145	TAL 582	TAL 82	A-309
		Visual ratings†			
TAL 1145	control	3	0	0	
TAL 582	control	0	3	0	
TAL 82	control	0	0	3	
A-309	control	0	0	0	
L-81-1	no inoc	2	0	0	
L-81-7	no inoc	2	1	0	
L-81-2	no inoc	1	1	1	
L-82-6	no inoc	3	0	0	
L-82-9	no inoc	3	0	1	
L-83-9	inoc	2	0	1	
L-94-2	inoc	2	1	1	
L-94-8	inoc	3	2	0	
L-101-4	inoc	0	3	0	
L-101-11	inoc	2	3	0	
L-102-3	no inoc	2	1	0	
L-102-7	no inoc	2	1	0	0

†Visual ratings were based on 0 = none, 1 = dim, 2 = medium, 3 - very bright fluorescing reaction.

[illegible] sia nictitans successfully nodulated Leucaena in the Gibson assemblies, while [illegible] from Indigofera hirsuta and Tephrosia hispiduia nodulated Leucaena in the growth pouches.

A preliminary Leonard jar study, briefly mentioned here, corroborated the results of the noduiation studies. It appeared that several indigenous Rhizobium strains isolated from native legumes were effectiveiy fixing nitrogen on L. ieucocephala grown on the acid soii (pH 4.2) medium used in the Leonard jar assemblies. In this study the piants grew pooriy from what appeared to be the sensitivity of the piant to the acid and Ai content of the soii medium. UF-1 and UF 2 accessions did, however, noduiate with Rhizobium strains isolated from Indigofera and Cassia. The internal pigmentation of these noduies was bright pink indicating the presence of leghaemogiobin and active nitrogen fixation.

Experiment 3 Acid and Aiuminum Toierance Studies

Acid-tripiate study

The data from the triplate agar study, presented in Table 4-4, indicate that Rhizobium strains isolated from the native legumes Galactia regularis, Indigofera hirsuta, and Tephrosia hispiduia and an introduced Leucaena sp. showed a marked degree of growth on yeast mannitol agar at pH 4.0. No growth of strains from other legumes was noted at pH 4.0, although aii strains grew weii or best at pH 6.0.

Table 4-4. Growth of selected strains of *Rhizobium* on yeast mannitol agar at various pH levels

Strain	Isolated from	Growth on acid triplates at pH	
		4.0	6.0
TAL 82	pure culture, NifTAL	0†	5
TAL 582	pure culture, NifTAL	0	5
TAL 1145	pure culture, NifTAL	5	5
SS-10	Leucaena spp	5	5
SS-39	Leucaena spp.	0	5
SS-46	Leucaena spp.	5	5
A-101	Aeschynomene americana	0	5
FP-102	Cassia nictitans	0	5
C-103	Crotalaria purshii	0	5
C-104	Galactia regularis	3	5
I-105	Indigofera hirsuta	3	5
S-106	Sesbania vesicaria	0	4
T-107	Tephrosia hispidula	3	5

†Visual ratings were based on a 0 to 5 scale, where 0 = no visible growth to 5 = large, gummy colonies after 18 days.

[illegible] studies, only TAL [illegible] to grow well at all three pH levels. Introduced Leucaena species [illegible] isolates also appeared to have some degree of acid tolerance, particularly SS 10 and SS-46. The absence of growth of several strains at pH 4.0 does not always indicate acid intolerance, since agar plates are artificial environments and do not necessarily mimic microbial growth under soil conditions. All strains except for TAL 1145, TAL 582, and TAL 82 were initially isolated from the roots of plants growing in acid (pH 4.2) soils. This in itself, suggests that tolerance to acid soil exists among these indigenous Rhizobium strains.

Broth culture

The four Rhizobium strains tested for growth in broth culture showed typical sigmoid curves of microbial growth (Figs. 4-3 and 4-4); however, rates of growth varied by treatment and strain. Growth rate was assessed by detectable turbidity expressed as optical density, pH change, and final cell population.

All strains appeared to grow well in Treatment 1 broth medium which contained yeast extract and had an initial pH of 6.3 (Fig. 4-3). The lag phase of strain SS-39 was slightly longer than the other strains; however, within 1 week the detectable turbidity of that strain in broth culture was equal to that of all other strains. Final pH

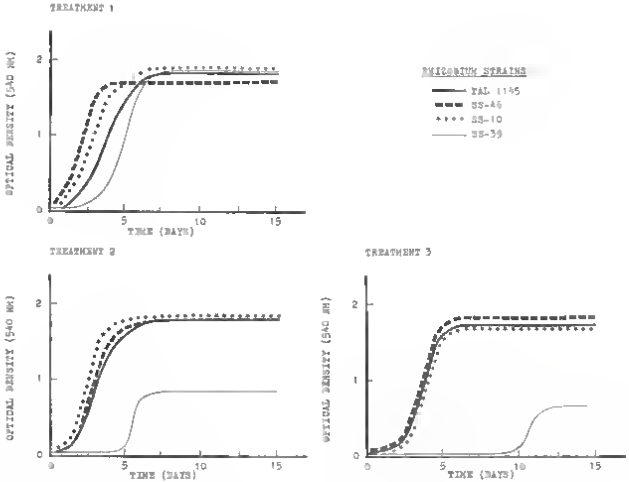

Fig. 4-3 Growth of Rhizobium strains in defined medium--Treatments 1, 2, and

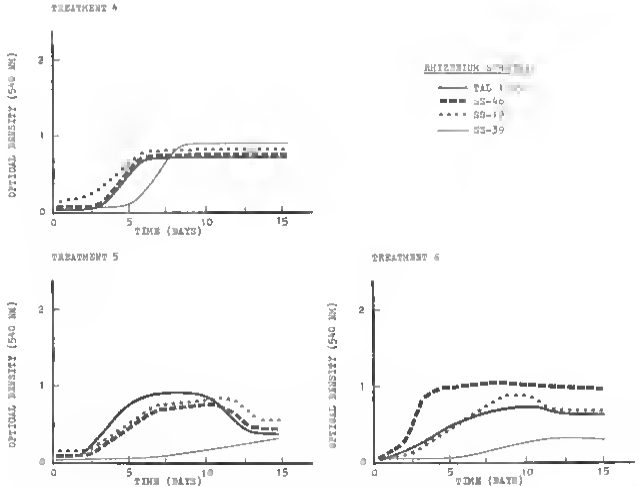

Fig. 4-4. Growth of Rhizobium strains in defined medium--Treatments 4, 5, and 6.

[illegible] lowered the pH of the original broth, and the [illegible] cell count was approximately 10^7 cells mL^{-1}.

[illegible] of the Rhizobium strains in Treatment 2 broth [illegible] which contained yeast extract but had a pH 4.5, was similar to strain growth in the same medium at pH 6.3, except for growth of strain SS 39. Strain SS-39 exhibited a long lag phase (Fig. 4-3), reduced turbidity, and a drop in pH from 4.5 at the start of the experiment to 4.0 (Table 4-[illegible].

Growth of strain SS-39 was also slowed and reduced in [illegible] saccharide production in the Treatment 3 broth culture [illegible] which Na glutamate was substituted for yeast extract (Fig. 4-3). The reason for the poor growth of the strain is [illegible] certain, but might be explained by a missing requirement supplied by the impurities in the yeast extract. Final pH of solutions changed only slightly, except in the case of SS-39, and population counts indicated that all strains multiplied to at least 10^6 cells mL^{-1}.

Although final pH and cell populations of all strains grown in Treatment 4 broth culture developed only slight pH changes and cell populations of 10^7 cells mL^{-1} (Table 4-5), optical densities of the strains were much lower than in the previous treatments (Fig. 4-4). Such reduction in turbidity may be due in part to the carbon source, Na glutamate, and/or the low levels of P added (5 μM KH_2PO_4).

appeared to be sensitive to both the acid pH of the medium and the level of Al. Strains TAL 1145, SS-46, and SS-10 caused the pH of the solution from approximately 4.5 to 6.2 while SS-39 did not appear to alter the pH significantly (Table [illegible]).

Final populations of all strains in both broth treatments were generally 10^7 cells mL^{-1}, but growth of strain SS-39 on yeast mannitol agar plates was slower (7 days) than the other strains (3 days), and colonies were pinpoint. The native Leucaena nodulating Rhizobium strains SS-46 and SS-10 appeared to grow as well as the introduced acid tolerant TAL 1145 strain in acidic and high Al media based on the turbidity of the media, pH change, and final cell population counts.

In general, strain SS 39 exhibited a longer lag phase than the three other strains, less turbidity (expressed as optical density), and no measurable alkali production compared to strains TAL 1145, SS-46, and SS-10, which significantly increased pH of the acid broth cultures. Although the growth of strain SS 39 on agar plates demonstrated the viability of the bacteria, colonies formed from cells grown in acidic and/or Al-containing medium were slow growing and pinpoint compared to the fast (3 day), gummy growth of the original culture. In comparison with

[illegible], SS-46, and [illegible] to be acid-sensitive.

Summary and Conclusions

Acid/Al tolerant Rhizobium strains that will nodulate Leucaena species are indigenous to Florida acid flatwoods, even in areas where Leucaena has never been grown. Such strains were isolated from nodules of native Indigofera, Tephrosia, and Cassia species but not from several other native leguminous species, suggesting that a degree of specificity among these indigenous cowpea miscellany Rhizobium exists. This specificity results in both an infective and effective nitrogen fixing symbiosis with Leucaena species.

CHAPTER V
[illegible]YDITY AND LIMING ON NODULATIO[illegible]
[illegible] OF *Leucaena* SPECIES IN FLORI[illegible]

Introduction

[illegible] are of interest in Florida because of [illegible] and biomass potential. Preliminary yield [illegible] the legume at Ft. Pierce and Gainesville [illegible] further evaluation of the *Leucaena* germplasm [illegible] in the state has merit. The plant adapts [illegible] variety of soil types, preferring well drained [illegible] neutral or alkaline soils (Oakes, 1968; Hutton, [illegible]). Cultivation in Florida, however, may be limited by [illegible] of *Leucaena* spp. on acid, flatwoods soils, [illegible] much of the pasture land in the state. [illegible] has suggested that it may be possible to [illegible] production on acid soils through breeding [illegible], and has developed some lines with improved [illegible]' tolerance (Hutton, 1981; 1982). Concurrent selection for acid tolerant *Rhizobium* species that nodulate *Leucaena* has also been underway (Halliday, 1981; Norris, 1972). In an effort to further evaluate some of these concepts, a field study and a greenhouse experiment were conducted to examine the effects of soil acidity on

of Rhizobium spscies.

Materials and Methods

Experiment 1

A field study was conducted on Myakka fine sand (sandy, siliceous, hyperthermic, Aeric Haplaquod), at the Beef Research Unit, Gainesville, Florida. Native soil pH was 4.2, extracted in distilled, deionized water (3.8 pH in 1 N KCl). Soil test analyses of samples taken prior to liming, in July, and December were conducted by the Soil Testing Laboratory, University of Florida, Gainesville. Results of the analysis are found in Table 5-1. Soil samples were a composite of ten random cores per subplot of 30 cm depth.

The field experimental design was a split plot with liming treatments (0, 2, and 6 Mg ha^{-1} lime) as main plots (24 m^2) and nitrogen treatments (Rhizobium inoculation, 200 kg ha^{-1} NH_4NO_3, and no inoculation) as subplots (8 m^2). Other fertilizer rates are listed in Table 5-2. Fertilizers were incorporated to a depth of 30 cm with a rototiller.

Six replications, three each of UF-1 and UF-2 ecotypes of Leucaena leucocephala (Lam) de Wit, were seeded at a rate of 25 g per 10-row subplot.

Subplots inoculated with Rhizobium spp. were seed-treated by coating with Pellnoc® (Nitragin Co., Inc., Clearwater, Florida) and a mixture of Rhizobium strains TAL

[illegible] of Nyakka soil

kg ha^{-1}		Mn			Zn			Cu			Fe			B		
		pH			DTPA											
			Ca													
0	Non-inoculated	4.7	5.3	4.9	97	119	121	23	49	44	1.1	1.0	9.0	11.[illegible]	[illegible]	11.1
	NH_4NO_3	4.6	5.2	4.9	83	110	125	21	46	41	[illegible]	9.5	8.0	12.7	[illegible]	[illegible]
	Rhizobium	4.6	5.0	4.8	93	119	130	25	55	41	1.1	10.8	9.8	10.[illegible]	[illegible]	[illegible]
2	Non-inoculated	4.8	5.5	5.4	83	435	508	39	61	59	2.1	10.8	13.8	18.0	[illegible]	[illegible]
	NH_4NO_3	4.9	5.4	5.4	63	484	480	41	61	60	2.1	13.1	12.5	18.[illegible]	[illegible]	[illegible]
	Rhizobium	5.0	5.0	5.3	71	545	475	17	72	62	2.[illegible]	14.3	12.1	16.7	[illegible]	[illegible]
6	Non-inoculated	4.9	7.0	7.1	67	1650	1627	16	95	86	2.1	19.9	18.8	14.3	[illegible]	[illegible]
	NH_4NO_3	5.0	7.0	6.7	60	1252	1005	13	75	69	2.0	16.5	17.3	15.3	[illegible]	[illegible]
	Rhizobium	4.8	7.3	7.0	80	1507	1291	18	97	87	2.2	22.0	21.0	19.[illegible]	[illegible]	[illegible]
		Al			Mn			N (%)								
0	Non-inoculated	58.0	78.7	75.3	0.[illegible]	0.16	[illegible]	7.80	7.[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	NH_4NO_3	48.9	72.0	68.3	0.13	0.60	[illegible]	9.3[illegible]	[illegible].01	[illegible]	[illegible]	[illegible]	[illegible].90	1.1[illegible]	[illegible]	[illegible]
	Rhizobium	61.3	81.3	76.0	0.[illegible]	0.44	[illegible]	2.58	[illegible].[illegible]7	1.66	[illegible].10	2.43	1.19	[illegible]	[illegible]	[illegible]
2	Non-inoculated	60.7	86.8	7[illegible].3	0.55	[illegible].66	[illegible]	2.65	1.[illegible]7	1.31	0.18	4.90	1.42	[illegible]	[illegible]	[illegible]
	NH_4NO_3	80.0	72.7	6[illegible].[illegible]	0.[illegible]	[illegible].71	[illegible]	[illegible].42	4.67	1.48	[illegible].18	5.23	1.50	[illegible]	[illegible]	[illegible]
	Rhizobium	80.0	58.3	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible]	[illegible].70	4.0[illegible]	1.49	[illegible].16	3.10	1.73	[illegible]	[illegible]	[illegible]
6	Non-inoculated	56.0	92.7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible].80	[illegible]	0.[illegible]2	0.17	12.87	1.46	[illegible]	[illegible]	[illegible]
	NH_4NO_3	54.7	96.7	[illegible]	0.[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible].67	[illegible].71	[illegible]	[illegible]	[illegible]
	Rhizobium	51.3	100.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible].84	[illegible].4[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 5 2. Fertilizer rate treatments for field p[illegible] experiment.

Element	Source	Rate ha^{-1} active	Plac[illegible]
Ca	$Ca(OH)_2$	1 Mg or 3 Mg	2 and 6 Mg ha^{-1} liming treatments
N	NH_4NO_3	200 kg	split application 100 kg ha^{-1} NH_4NO_3 treatments
Micro nutrient frit	FTE 503®	30 kg	all plots
K	KPO_4	400 kg	split application (200 kg ha^{-1} all plots)
Mg	$MgSO_4$	100 kg	all plots
P	P_2O_5 (Triple Super Phosphate)	40 kg	all plots

NifT[illegible] Viabili[illegible] Inoculum was tested prior to field [illegible] by dilu[illegible]es and plate counts es outli[illegible] Vincent (1970). [illegible] eeds were controlled by selec[illegible] on of glyphosate at a rate of 1 L he^{-1}.

Th[illegible] response variables measured were plant yield, plant number, module number, and module dry weight recorded on 4 Oct. 1981 and 11 Aug. 1982.

Data analyses were performed using SAS at NERDC. Treatment means were tested using the Least Significant Difference (LSD) procedure at P = 0.05.

Experiment 2

This experiment was designed to screen for acid soil tolerance in 75-day old Leucaena seedlings grown in Conetainers®. Conetainers® (Supercell, 150 cc volume) were arranged in a split-split plot design with three soil types as the main plots, two liming treatments as subplots, and 98 Leucaena genotypes as sub-subplots. The experiment had 7 replications. The seedlings were grown under a shade house with a 30% sunlight reduction, at Gainesville, Florida, from 6 May. to 20 Oct. 1982. The three soils used in this experiment are described in Table 5-3.

'Liming' treatments consisted of no amendments or additions of 1 g $CaCO_3$ and 1 g dolomitic lime kg^{-1} soil for both Bladen and Myakka soils and 3.44 g gypsum ($CaSO_4$ $2H_2O$)

Table 5-3. Mineral and pH analysis and soil classification of Bladen, Myakka, and Rockdale soils

Soil type	Liming treatment	Soil pH		Double acid extractable nutrient				
		H_2O	KCl	Al	Ca	K	Mn	
				ppm				
Bladen	nonlimed	4.7	4.0	216	72	4	0.24	
	limed	7.3	6.8	188	940	8	28.40	
Myakka	nonlimed	4.1	3.2	16	132	12	1.08	
	limed	6.2	6.1	32	560	16	27.20	
Rockdale	nonlimed	7.6	7.3	trace	7600	88	0.36	
	limed	7.4	7.3	trace	8000	104	1.52	

Soil Series	Taxonomic class
Bladen	Clayey, mixed, thermic Typic Albaqults
Myakka	Sandy, siliceous, hyperthermic, Aeric Haplaquods
Rockdale	Loamy, siliceous, hyperthermic, shallow Lithic Udorthents†

†Personal communication with Adam Hyde, Soil Conservation Service, Gainesville, Flori

micronntrient fri[illegible]
containing 18.0% Fe, 7.5% Mn, 7.0% Zn, 3.0% B,
[illegible] applied to all soils at a rate of 20 mg kg^{-1}
soil. All soils were then fumigated with methyl bromide
(Dowfume MC-2, Dow Chemical Co.) at a rate of 680 g per 2.3
m^3, 2 weeks prior to planting.

Ninety-eight Leucaena genotypes of diverse origin were selected for evaluation (Table 5-4). All seeds were hand scarified, then surface-sterilized by immersion in 95% EtOH for 30 sec followed by 0.2% $HgCl_2$ for 3 min, as outlined by Vincent (1970), and germinated at approximately 25°C for 72 hrs. Seedling roots were dipped into a mixed strain peat-based Rhizobium slurry containing strains TAL 1145, TAL 582, and TAL 82 and transplanted immediately following inoculation. The seedlings were watered with tap water when natural rainfall was inadequate to maintain proper soil moisture. Plants were grown from 8 August until 22 October.

Data were analyzed using SAS at NERDC at the University of Florida. Duncan's New Multiple Range Test was utilized for comparisons of plant accession means within individual treatments.

Results and Discussion

Experiment 1

Liming at the 2 and 6 Mg ha^{-1} level significantly increased plant number, plant yield, nodule number, and

[illegible] of PI [illegible] and origin o[illegible]
[illegible]ersions [illegible] experiment.

[illegible]	[illegible]	Ori[illegible]
[illegible]	[illegible]	Florida
[illegible]	[illegible]	Florida
[illegible]	[illegible]	U.S. Virgin Islands
[illegible]	[illegible]	U.S. Virgin Islands
[illegible]	[illegible]	U.S. Virgin Islands
[illegible]	[illegible]	Colombia
[illegible]	[illegible] leucocephala x	Colombia
	[illegible] pulverulenta	
[illegible]	L. leucocephala x	Colombia
	[illegible] pulverulenta	
[illegible]	L. leucocephala x	Colombia
	L. pulverulenta	
[illegible]	L. pulverulenta x	Colombia
	L. leucocephala	
CIAT 22/5-6	L. diversifolia	Colombia
CIAT 26/78-49	L. diversifolia	Colombia
CIAT 30/4-11	L. diversifolia	Colombia
CIAT "Gigante" 78-1[illegible]	L. leucocephala	Colombia
188810	L. leucocephala	Philippines
237147	L. leucocephala	Costa Rica
241167	L. leucocephala	Philippines
247682	L. leucocephala	Belgian Congo
[illegible]6635	L. esculenta	Mexico
[illegible]7170	L. leucocephala	South Africa
[illegible]180	L. leucocephala	India
279577	L. leucocephala	Taiwan
281605	L. leucocephala	U.S.A.
281606	L. leucocephala	Colombia
281627	L. leucocephala	Australia
281636	L. leucocephala	Tanganyika
281766	L. leucocephala x	New Guinea
	L. pulverulenta	
281767	L. leucocephala x	New Guinea
	L. pulverulenta	
281769	L. leucocephala x	New Guinea
	L. glabrata	
281771	L. leucocephala	New Guinea
281772	L. leucocephala	New Guinea
281774	L. leucocephala	New Guinea
281775	L. leucocephala	New Guinea
281777	L. leucocephala	New Caledonia
281778	L. leucocephala	New Caledonia
281779	L. leucocephala	New Caledonia
281780	L. leucocephala	Puerto Rico
281781	L. leucocephala	Ceylon (Sri Lanka)

	[illegible]cocephalu	Ghana
	[illegible]tocephala	Sierra L[illegible]
84	[illegible]cocephala	Senegal
96	[illegible]cocephala	Philippines
04	[illegible]cocephala	Colombia
405	[illegible]a	Honduras
38	[illegible]cocephala	Colombia
0	[illegible]ularum	Australia
	[illegible]cocephala	Australia
	[illegible]cocephala	Australia
	[illegible]cocephala	Australia
	[illegible]cocephala	Australia
	[illegible]cocephala	Australia
	[illegible]cocephala	Australia
	[illegible]cocephala	Australia
	[illegible]cocephala	Australia
	[illegible]cocephala	Australia
	[illegible]cocephala	Australia
	[illegible]cocephala	Australia
	[illegible]cocephala	Australia
	[illegible]cocephala	Mexico
	[illegible]cephala	Taiwan
	[illegible]cephala	Brazil
28	[illegible]ulata	Mexico
786	[illegible]cephala	Ivory Coast
28800	[illegible]cephala	U.S.A.
28800	[illegible]cephala	U.S.A.
288.0	[illegible]cephala	U.S.A.
2880	[illegible]cephala	U.S.A.
288	[illegible]cephala	U.S.A.
28.00	[illegible]cephala	U.S.A.
	[illegible]cephala	U.S.A.
	[illegible]cephala	U.S.A.
	[illegible]cephala	Bolivia
	[illegible]cephala	Taiwan
	[illegible]cephala	Taiwan
	[illegible]cephala	Taiwan
	[illegible]cephala	Taiwan
	leucocephala	Taiwan
L.	leucocephala	South Africa
L.	leucocephala	South Africa
L.	leucocephala	Argentina
L.	leucocephala	Sierra Leone
L.	leucocephala	Colombia
L.	leucocephala	Venezuela
L.	leucocephala	Nicaragua

Table 2 [illegible]

Accession	Species	Origin
311513	[illegible] leucocephala	Brazil
317908	[illegible] leucocephala	U.S.A.
317909	[illegible] leucocephala	U.S.A.
317910	[illegible] leucocephala	U.S.A.
317911	[illegible] leucocephala	U.S.A.
317912	[illegible] leucocephala	U.S.A.
367835	[illegible] leucocephala	Argentina
370749	[illegible] leucocephala	U.S.A.
384516	[illegible] leucocephala	Bahamas
384517	[illegible] leucocephala	Bahamas
443604	[illegible] leucocephala	Mexico
443479	[illegible] leucocephala	El Salvador
443559	[illegible] leucocephala	Mexico
443700	[illegible] leucocephala	Mexico

[illegible] eight in 1984, and plant number and nodule number in 1982, when compared to plants grown on the nonlimed acid soil (Table 5-5). In 1981, the year of establishment, 6 Mg of lime increased plant number and yield compared to the 2 Mg ha^{-1} treatment, although nodule number and nodule dry weights were not apparently affected. In 1982, plants which had successfully overwintered were sampled. Although removal of plants for yield data in 1981 for yield data and winter losses had somewhat reduced plant numbers, significant increases in plant dry matter yield, nodule numbers and nodule dry weights of plants grown on the 6 Mg ha^{-1} limed soils were apparently greater than those of plants grown on the 2 Mg ha^{-1} limed plots. Plant numbers were significantly reduced only in the nonlimed plots.

There were no significant interaction effects of liming and nitrogen treatments. In 1981, additions of ammonium nitrate did not significantly increase plant numbers or yields obtained from the nontreated or the Rhizobium inoculated plots (Table 5-6). Nodulation occurred on plants grown on both inoculated and noninoculated plots. This indicates the presence of indigenous Rhizobium species capable of nodulating Lencaena. Although significantly fewer nodules were also found on plants grown on the non-limed, noninoculated plots, as shown in Chapter IV, a degree of acid/aluminum tolerance exists in these native Rhizobium spp. Rhizobium inoculation significantly increased nodule

Table [illegible]. Effects of li[illegible] and no[illegible] on Leucaena le[illegible] r a Myako sandy, acid fla[illegible]

Liming level	Plants	Dry matter yield	Nodules	Nodule dry wt
Mg ha^{-1}	no ha^{-1}	kg ha^{-1}	no plant^{-1}	g plant^{-1}
		1981		
6	38,750 a*	1761 a	30 a	0.128 a
2	27,900 b	788 b	36 a	0.170 a
0	13,750 c	159 c	5 b	0.008 b
		1982		
6	5,850 a	404 a	45 a	0.062 a
2	4,150 a	114 b	24 b	0.024 b
0	400 b	2 b	9 c	0.01o b

*Means in the same column not followed by the same letter are significantly different at the 0.05 level.

Table [illegible]. Effects of nitrogen fertilization and inoculation with Rhizobium [illegible] on plant growth and nodulation of Leucaena leucocephala grown on a Myakka fine sandy, acid flatwoods soil

Treatment	Plants	Dry matter yield	Nodules	Nodule dry wt.
Mg ha^{-1}	no ha^{-1}	kg ha^{-1}	no plant^{-1}	g plant^{-1}
		1981		
Rhizobium Inoc.	23,750 b*	913 a	36 a	0.135 a
NH_4NO_3	27,100 ab	848 a	16 b	0.077 a
Control	29,600 a	937 a	18 b	0.094 a
		1982		
Rhizobium Inoc.	1,650 a	101 a	26 a	0.043 a
NH_4NO_3	4,150 a	243 a	24 a	0.024 a
Control	4,600 a	175 a	26 a	0.035 a

*Means in the same column not followed by the same letter are significantly different at the 0.05 level.

number, but [illegible] y weight, [illegible]en compare[illegible] [illegible] nonincoculated plots. There were fewer bi[illegible] and more lobed nodules on nonincoculated plants th[illegible] nococulated ones

Plant number, plant dry matter yield, nodule number, and nodule dry weight did not appear to be affected by Rhizobium inoculation or ammonium nitrate treatments in 1982 when compared with the control plants (Table 5-6). Some of the variation in plant stand and dry matter yield between nitrogen treatments may be explained by the luxuriant plant growth in several 6 Mg ha^{-1} limed plots.

The results of this 2 year experiment appear to support the conclusion that Leucaena is rather acid and aluminum sensitive. While "acid soils" do vary in pH and amount of exchangeable calcium and aluminum, these data suggest that these two Florida ecotypes of L. leucocephala (UF 1 and UF-2) have markedly decreased productivity when grown on native, acid flatwoods soil. These results further emphasize the need for selection and breeding for more acid tolerant Leucaena germplasm.

Experiment 2

The Conetainer® flats provided a practical approach for the large scale germplasm evaluation completed in this study. In 1983, 98 150 cc size super cell cones cost approximately $11.00 including the supportive rack. In

competit[illegible] and near [illegible]
ater, t[illegible] ight. Coneta m[illegible] also is
[illegible] nodul[illegible] c[illegible] a) soil vo[illegible]
[illegible] b) the soil can be uniformly inocula[illegible]
[illegible] given depth with a "repeating pipet," and c) root[illegible] on ainer bound and intact root systems c[illegible] be easily [illegible] of the cones for observation.

Anal[illegible] data from this experiment revealed a high[illegible] significant ([illegible]) three way interaction among plant genotype, soil type, and lime treatment. Therefore, it as necessary to examine the simple effects to aid in interpretation of the data.

Acid soil is a poorly defined term, related in part to the amount of exchangeable aluminum in a soil, and also to its organic matter and salt content (Kamprath, 1972). Both Blanton and Nyakka soils were used in this study as representatives of acid soil types common in Florida. Each differs in percent base saturation and exchangeable calcium and aluminum, as well as soil structure and texture. The Pocrdole soil, a calcareous soil type, was also used in the study to provide a comparison of the growth of Leucaena on a near neutral pH Florida soil with growth of the plant on both acid soil types.

The top 10% of all accessions for plant dry weight, shoot height and nodule number are listed by soil type and lime treatment in Tables 5-7, 5-8, and 5-9.

[illegible]

	Plant dry weight			
	Limed		Unlimed	
	g			
	Myakka			
[illegible]	0.50 a*	CIAT 78 15/11	0.35 a	
[illegible]	0.37 a	UF-1	0.29 b	
[illegible]	0.34 ab	286295	0.25 bc	
[illegible]	0.33 ab	263695	0.25 bc	
[illegible]	0.32 ab	CIAT 22/9 7	0.24 bc	
[illegible]	0.31 ab	262473	0.23 c	
[illegible]	0.30 ab	262405	0.22 c	
[illegible]	0.30 ab	UF 2	0.21 c	
[illegible]	0.29 ab	CIAT 17 5/2 27	0.21 c	
[illegible]	0.11 b	CIAT 25/5-6	0.20 c	
	Rockdale			
[illegible]	0.80 a	UF-1	0.85 a	
[illegible]	0.77 a	UF-2	0.83 ab	
[illegible]	0.73 ab	CIAT 22/9 7	0.75 abc	
[illegible]	0.73 ab	CIAT 78-15/11	0.73 bcd	
[illegible]	0.71 ab	CIAT 25/5-6	0.65 cde	
CIAT [illegible]	0.70 bc	286295	0.63 de	
286295	0.63 bc	CIAT 17-5/2 27	0.59 e	
VI-1	0.68 bc	CIAT 19-12/3 3	0.56 e	
263695	0.59 c	CIAT 30/4 11	0.54 e	
CIAT 20-11/1 [illegible]	0.59 c	263695	0.53 a	
	Bladen			
CIAT 22/9-7	0.61 a	UF-1	0.36 a	
CIAT 78 15/11	0.50 a	CIAT 78 15/11	0.36 a	
UF-1	0.49 a	262405	0.30 b	
UF-2	0.41 b	286295	0.30 b	
286295	0.37 bc	CIAT 22/9-7	0.29 b	
VI-1	0.37 bc	UF 2	0.27 b	
CIAT 17-5/2-7	0.37 bc	CIAT 17-5/2-27	0.26 b	
CIAT 19-12/3 3	0.37 bc	CIAT 30/4 11	0.25 b	
443700	0.33 cd	VI-1	0.25 b	
298622	0.30 d	CIAT 19-12/3-3	0.25 b	

*Means in the same column not followed by the same letter are significantly different at the 0.05 level.

Table [illegible]. Height of Leucaena acce[illegible], Rockdale, and Bladen soils.

	Shoot height			
	Limed			
	mm			
Myakka				
[illegible]	15.13	a*	CIAT 25/5-7	[illegible] a
[illegible]	13.64	ab	VI 3	[illegible] ab
[illegible]	12.79	ab	CIAT 16/1-7	[illegible]
[illegible]	12.51	b	CIAT 78-15 11	[illegible]
[illegible]	12.34	b	282405	[illegible]
[illegible]	11.99	b	CIAT 26/78-49	[illegible]
[illegible]	11.87	b	286295	[illegible]
[illegible]	11.83	b	UF-1	[illegible]
[illegible]	11.74	b	288011	[illegible]
[illegible]	11.73	b	CIAT 19-12/3-3	[illegible]
Rockdale				
[illegible]	14.96	a	CIAT 16/1 7	[illegible]
[illegible]	12.80	b	CIAT 25/5-6	[illegible].3 ab
[illegible]	12.39	b	288001	11.03 abc
[illegible]	11.73	b	CIAT 26/78-49	11.00 bc
[illegible]	11.34	b	UF-2	11.08 bc
[illegible]	11.34	b	UF-1	10.67 c
[illegible]	11.34	b	CIAT 30/4-11	10.61 c
[illegible]	11.14	b	288011	10.40 c
[illegible]	11.13	b	CIAT 78-15/11	10.36 c
[illegible]	10.91	b	CIAT 22/9-7	10.30 c
Bladen				
[illegible]	10.73	a	CIAT 78-15/11	6.07 a
[illegible]	9.46	ab	CIAT 16/1-7	5.49 ab
[illegible]	8.71	bc	286295	4.93 bc
[illegible]	8.10	bc	282405	4.93 bc
[illegible]	7.89	bc	UF 2	4.89 bc
[illegible]	7.67	bc	UF-1	4.77 bc
[illegible]	7.49	bc	288011	4.71 bc
[illegible]	7.25	c	443559	4.58 c
[illegible]	7.09	c	370749	4.56 c
[illegible]	7.06	c	281780	4.51 c

[illegible] the same column not followed by the same letter are [illegible]ntly different at the 0.05 level.

[illegible] 4. Nodulation of Leucaena accessions grown on Myakka, Rockdale, and Sladen soils.

Limed	Nodules	Unlimed
	no plant^{-1}	
	Myakka	
[illegible] 7	39.29 a*	CIAT 16/1-7	20.86 a
CIAT [illegible] /-27	39.14 a	CIAT 26/78-49	18.00 a
2862	34.00 ab	CIAT 25/5-6	17.43 a
UF-2	33.29 ab	UF-2	12.14 b
CIAT 19-1-/3 5	30.14 ab	286295	12.00 b
CIAT 25/5-6	28.14 ab	286248	10.57 b
CIAT 26/78-49	27.86 ab	282471	9.86 b
VI-3	27.00 b	263695	9.71 b
282471	26.86 b	304650	9.57 b
VI-1	26.71 b	CIAT 30/4-11	9.29 b
	Rockdale	
CIAT 25/5 6	40.14 a	CIAT 25/5-6	38.43 a
CIAT 16/1 7	24.29 b	CIAT 16/1-7	18.71 b
CIAT 26/78-49	19.71 bc	CIAT 26/78-49	13.86 c
292345	14.43 cd	CIAT 22/9-7	7.29 d
281772	11.71 d	292345	5.57 d
282692	11.71 d	288004	5.29 d
CIAT 17-5/2 27	11.43 d	308544	5.14 d
282471	11.29 d	282474	4.71 d
281605	10.57 d	282692	4.71 d
288011	10.57 d	286295	4.57 d
	Sladen	
UF-2	9.57 a	UF-2	19.86 a
CIAT 26/78-49	9.57 a	CIAT 16/1-7	12.29 b
CIAT 22/9-7	8.86 a	CIAT 25/5-6	11.71 b
CIAT 25/5-6	8.00 a	263695	6.71 c
CIAT 76-15/11	7.43 ab	282405	6.71 c
CIAT 16/1-7	7.00 ab	CIAT 26/78-49	6.57 c
279180	5.29 b	308544	6.57 c
UF 1	5.14 b	295362	6.43 c
443479	5.14 b	298622	6.29 c
CIAT 17-5/2 27	4.86 b	CIAT 22/9 7	5.29 c

*Means in the same column not followed by the same letter are significantly different at the 0.05 level.

[illegible] plant dry weight, shoot height, and nodule number when grown on either acid Bladen or Myakka soil. Several accessions did, however, have a higher relative ranking on the acid soils than on the limed soil. It was common to find that rankings of accessions for a given response variable changed in order from soil to soil and among the lime treatments.

One example of this is PI 282405, which ranked in the top 10% of all accessions in plant dry weight and shoot height for plants grown on acid Myakka and Bladen soils. This same accession ranked 24th and 12th for dry weight and 50th and 33rd for shoot height, respectively, when grown on limed Myakka and Bladen soils. A similar change in ranking also occurred among the accessions for nodulation under acid soils conditions. PI 263695 ranked in the top 10% of all accessions for nodulation under acid soil conditions, yet dropped in ranking to positions 37 and 42 on the limed Myakka and Bladen soils, respectively.

Table 5-10 lists superior acid soil-tolerant plant genotypes derived by imposing a 10% selection pressure on the accessions based on their ranking for all response variables studied. The selection was then further divided into two categories, genetic vigor and acid tolerance. The genetic vigor class includes those accessions that exhibited superior growth and/or nodulation (top 10%) on both acid and

nt Leucaena acc

	Shoot height		Nodule num	
Rank	Rank	Accession	Rank	Accession
1	1	CIAT 16/1 7	1	CIAT 16/1 7
1	2	282405	2	UF-2
2	3	286295	3	CIAT 25/5-6
3	4	UF 1	4	CIAT 26/78-49
3	5	288011	5	263695
4				
5				

	Genetic vigor	Acid tolerance
	CIAT 78-15/11	CIAT 22/9-7
	1	282405
	86295	
	UF-2	
	CIAT 17-5/2-27	
	286 95	CIAT 16/1 7
	UF-1	282405
	288011	
	CIAT 16/1 7	263695
	UF-2	
	CIAT 25/5-6	
	CIAT 26/78-49	

limed Myakka and Bladen so[illegible] class includes only those [illegible] ranked relatively higher on the unlimed treat[illegible] limed treatment of Bladen and Myakka soils.

Superior acid soil to[illegible] genotypes included several of Dr. J. E. Hutton's [illegible] which had grown well on Carimagua oxisols in Colombia. [illegible] lines were derived from inter- and intra-specific [illegible] hybridization among several Leucaena species. CIAT 78 16/1 is L. leucocephala, CIAT 16/1-7 is L. shannoni, and both CIAT 25/5-6 and CIAT 25/78-49 are L. diversifolia. Leucaena leucocephala crossed with L. pulverulenta produced acid soil tolerant CIAT 17-5/2-27 and CIAT 22/9-7. Several accessions from the USDA Plant Introduction Collection that also performed well on Florida acid soils include PI 286295, PI 282405, PI 288011, and PI 62695. Plant introduction (PI) 282405, a Honduran ecotype, is of unknown species, and PI 263695 (K8), from Mexico, is L. esculenta. Plant introduction (PI) 286295 and PI 288011 are both L. leucocephala. UF-1 and UF-2 are two Florida Leucaena ecotypes of unknown origin which have been submitted to the USDA Plant Introduction Collection, and are believed to be L. leucocephala.

Several conclusions can be drawn from this study: a) plant genotypes vary in response to growth on different acid soils, b) some degree of acid/aluminum tolerance exists among the Leucaena germplasm, and c) genetic vigor can be

[redacted] Recommended genotypes to [redacted] on acid soils include ecotypes UF-1 and UF 2, [redacted], PI 288011, PI 286295, and CIAT lines 78-15/11, 22/[redacted]-1, [redacted]-7/2-27, and 16/1-7.

General Summary and Conclusions

Field- and Conetainer®-grown Leucaena showed a marked sensitivity to acid soils. Yet some degree of tolerance among plant genotypes does exist. Several genotypes can be recommended for acid soils; but, no accession among the Leucaena species studied reached full production potential on those soils. Further selection among accessions for acid/alumium tolerance has questionable merit, yet some general improvement over existing cultivars may be possible through plant breeding with acid/aluminum tolerant genotypes.

CHAPTER VI
SCREENING FOR Al TOLERANCE IN *Leucaena* SPECIES

Introduction

[illegible] greatly affects the growth of [illegible] growth suppression of the plant [illegible] soil may be due, in part, to toxic Al [illegible] (Hutton, 1980). *Leucaena* genotypes tolerant to acid [illegible] and high Al levels may offer a solution. The main effect of Al on plant growth is reduced or distorted root development. Thus, by evaluating root growth parameters, genotypic differences in response to Al in solution may be identified.

The objectives of these solution culture experiments were a) to determine the effect of varying levels of Al and Ca on seedling root growth and development, and b) to determine the relative Al tolerance expressed by root growth among a number of *Leucaena* genotypes of diverse origin.

Materials and Methods

Experiment 1

Five genotypes of *Leucaena* species (UF-1, UF 2, Vl-1, PI 251606, and PI 308544) were used in a solution culture study conducted in the laboratory to determine the

[illegible] of [illegible] the [illegible] growth [illegible] of the five genotypes [illegible] with 95 [illegible] 30 sec, transferred to 0.2% $HgCl_2$ [illegible] min and then [illegible] six times in sterile, distilled, deionized [illegible] (Vincent, 1970). Seeds were then germinated at 25°C on [illegible] filter paper in sterile Petri dishes. After a 72 [illegible] germination period, root length from crown to the tip of the tap root was measured and the seedlings were then placed in plastic freezer container assemblies (Fig. 1). Roots were submerged in the treatment solutions. Twenty different solution combination treatments of four Ca concentrations (5, 10, 20 and 40 ppm) supplied as $CaCl_2 \cdot 2H_2O$ and five Al concentrations (0.0, 0.5, 1.0, 2.0, and 4.0 ppm) supplied as $AlCl_3 \cdot 6H_2O$ were used. The pH of all treatments was adjusted to 4.6 by adding either 0.1 N HCl or 1.0 N NaOH as required. The solutions were aerated by bubbling compressed air through Tygon® (Fisher Scientific Co.) tubing into the containers. The solutions were maintained at ambient laboratory temperature. Root length of all seedlings was remeasured 120 hrs after being placed in the solutions. Solution pH was determined at the termination of the experiment.

The experimental design used was a split-split plot with Ca levels as the main plots, Al levels as the subplots, and plant accessions as the sub-subplots. Five replications were used. Duncan's Multiple Range Test was used to rank

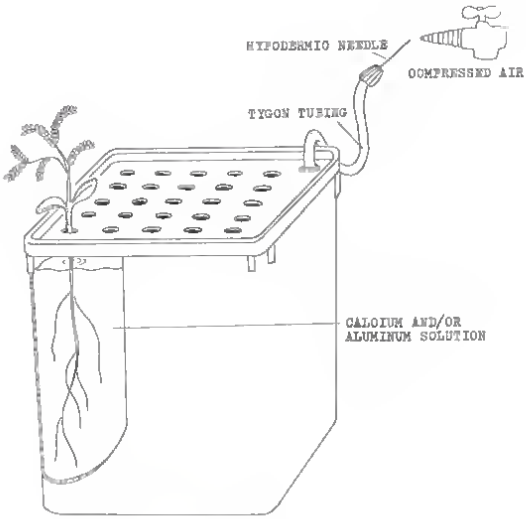

Fig. 6-1. Solution culture apparatus

the varietal mean adjusted root growth ([illegible] adjusted root growth was derived from a modification of the formula for relative root extention rate (RER) proposed by [illegible] et al (1965). The ARG was assessed by the following formula

$$ARG = \frac{t_1 - t_0}{t_0}$$

where t_0 and t_1 are the initial and final length of the roots in mm, respectively. Data were analyzed using SAS on the IBM DC, at the University of Florida.

Experiment 2

In this experiment, variation among 100 Leucaena accessions (Table 6.1) for tolerance to Al in solution was studied. Seedling sterilization, germination, and growth conditions were as outlined in Experiment 1 except that final root lengths were determined at 96 hr after being placed in solution. Based on the results of Experiment 1, a level of 5 ppm Ca from $CaCl_2 \cdot 2H_2O$ in combination with four Al concentrations (0.0, 0.5, 0.1, 0.2, and 0.4 ppm) from $AlCl_3 \cdot 6H_2O$ were imposed as treatments.

The experimental design was a split plot with Al levels as the main plot and plant accessions as the subplot factors. Five replications were used. Duncan's Multiple Range Test was used to rank the varietal mean adjusted root growth as outlined in Experiment 1.

[illegible] Leucaena

[illegible]	[illegible]	Origin
[illegible]	L. [illegible]	Florida
[illegible]	L. [illegible]	Florida
[illegible]	L. [illegible]	U.S. Virgin Islands
[illegible]	L. [illegible]	U.S. Virgin Islands
[illegible]	L. [illegible]	U.S. Virgin Islands
[illegible]	L. [illegible]	Philippines
[illegible]	L. [illegible]	Costa Rica
[illegible]	L. [illegible]	Philippines
[illegible]	L. [illegible]	Belgian Congo
[illegible]	L. [illegible]	Mexico (Original Seed)
[illegible]	L. [illegible]erulenta	Mexico
[illegible]	L. [illegible]ucocephala	South Africa
[illegible]	L. [illegible]cocephala	India
[illegible]	L. [illegible]cocephala	Taiwan
281[illegible]	L. [illegible]ucocephala	U.S.A.
281[illegible]	L. [illegible]cocephala	Coombia
281[illegible]	L. [illegible]cocephala	Australia
2816[illegible]	L. [illegible]ocephala	Tanganylka
28174[illegible]	L. [illegible]cocephala x [illegible]erulenta	New Guinea
28176	L. [illegible]ocephala x [illegible]erulenta	New Guinea
281769	L. [illegible]cocephala x [illegible]orata	New Guinea
281771	L. [illegible]eucocephala	New Guinea
281772	L. [illegible]ucocephala	New Guinea
281774	L. [illegible]cocephala	New Guinea
281775	L. [illegible]cocephala	New Guinea
281777	L. [illegible]cocephala	New Caledonia
281778	L. [illegible]cocephala	New Caledonia
281779	L. leucocephala	New Caledonia
281780	L. leucocephala	Puerto Rico
281781	L. leucocephala	Ceylon
281782	L. leucocephala	Ghana
281783	L. leucocephala	Sierra Leone
281784	L. leucocephala	Senegal
282396	L. leucocephala	Philippines
282404	L. leucocephala	Colombia
282405	L. sp.	Honduras
282458	L. leucocephala	Colombia
282460	L. insularum	Australia
282462	L. leucocephala	Australia
282463	L. leucocephala	Australia

[illegible]	L. [illegible]	[illegible]
[illegible]	L. [illegible]	Australia
[illegible]	L. leu[illegible]	Australia
[illegible]	L. leuc[illegible]	Australia
[illegible]	L. leuc[illegible]	Australia
[illegible]	L. leuco[illegible]	Australia
[illegible]	L. leu[illegible]	Australia
[illegible]	L. leuco[illegible]	Australia
[illegible]	L. [illegible]	Australia
[illegible]	L. [illegible]	Australia
[illegible]	L. [illegible]	Australia
[illegible]	L. [illegible]	Australia
[illegible]	L. [illegible]	Mexico
[illegible]	L. [illegible]	Taiwan
[illegible]	L. leucocephala	New Caledonia
[illegible]	L. lanceolata	Mexico
[illegible]	L. leucocephala	Ivory Coast
[illegible]	L. leucocephala	U.S.A.
[illegible]	L. leucocephala	U.S.A.
[illegible]	L. leucocephala	U.S.A.
[illegible]	L. leucocephala	U.S.A.
[illegible]	L. leucocephala	U.S.A.
[illegible]	L. leucocephala	U.S.A.
[illegible]	L. pulverulenta	U.S.A.
[illegible]	L. leucocephala	U.S.A.
[illegible]	L. leucocephala	Bolivia
[illegible]	L. leucocephala	Taiwan
[illegible]	L. leucocephala	Taiwan
[illegible]	L. leucocephala	Taiwan
[illegible]	L. leucocephala	Taiwan
[illegible]	L. leucocephala	South Africa
[illegible]	L. leucocephala	South Africa
[illegible]	L. leucocephala	Argentina
[illegible]	L. leucocephala	Sierra Leone
30[illegible]	L. leucocephala	Colombia
30d[illegible]	L. leucocephala	Venezuela
3111[illegible]	L. leucocephala	Nicaragua
311[illegible]	L. leucocephala	Brazil
3171[illegible]	L. leucocephala	U.S.A.
3170[illegible]	L. leucocephala	U.S.A.
317910	L. leucocephala	U.S.A.
317911	L. leucocephala	U.S.A.
3[illegible]631	L. retusa	U.S.A.
3[illegible]7835	L. leucocephala	U.S.A.
[illegible]67835	L. leucocephala	Argentina
370749	L. leucocephala	U.S.A.
384516	L. leucocephala	Bahamas

Acce		
384517	L. leucocephal	
404037	L. leucocephal	U.S.
414771	L. leucocephal	Aust
443548	L. esculata	Mexic
443559	L. leucocephala	Mexic
443730	L. shannoni	Mexico
443735	L. shannoni	Mexico
443735	L. shannoni	Mexico
443737	L. shannoni	Mexico
460766	L. leucocephala	Togo
460 07	L. leucocephala	Ghana
460 65	L. leucocephala	Congo
460	L. leucocephala	Senegal
460710	L. leucocephala	Senegal

Experiment 3

Variatio [illegible] in solutio[illegible]d ed among 25 selec[illegible]sions, some of [illegible]n were thought to ha[illegible] Al tolerance. The plant accessions incl[illegible] breeding lines and interspecific [illegible] from E. M. Hutton (CIAT, Cali, Colombia[illegible] greenhouse and field Al tolerance (Tabl[illegible] preparation and solution treatments [illegible]re as outli[illegible]periment 1, except that final root lengths were [illegible] 72 hrs after seedlings were placed in solution. [illegible]mental design and data analysee were as outlined in Experiment 1.

Results and Discussion

Experiment 1

Adjusted root growth (ARG) is an expression of the growth of the root over a period of time. Differences in unequal initial root lengths are adjusted for by the formula, and thus genetic variability for root growth in Al solution among the accessions tested should be more accurately evaluated.

Since there were significant three-way interactions among genotypes, Ca and Al levels it was necessary to make only simple comparisons among accessions at each level of Ca and Al (Table 6 3).

Table [illegible]. List of PI number, species and origin of Leucaena accessions used in Experiment 3.

Accession	Species	Origin
UF 1	L. leucocephala	Florida
UF 2	L. leucocephala	Florida
VI-1	L. leucocephala	U.S. Virgin Islands
VI 3	L. leucocephala	U.S. Virgin Islands
263695	L. leucocephala	Mexico (Original Seed)
279180	L. leucocephala	India
281606	L. leucocephala	Colombia
282464	L. leucocephala	Australia
282692	L. leucocephala	Mexico
286248	L. lanceolata	Mexico
292345	L. leucocephala	Bolivia
295363	L. leucocephala	Taiwan
295364	L. leucocephala	Taiwan
308544	L. leucocephala	Colombia
321631	L. retusa	U.S.A.
414771	L. leucocephala	Australia
CIAT 78-15	L. leucocephala	Colombia
CIAT 16/1 7	L. shannoni	Colombia
CIAT 17-5/2-27	L. leucocephala x L. pulverulenta	Colombia
CIAT 19-12/3-3	L. leucocephala x L. pulverulenta	Colombia
CIAT 20-11/1 21	L. leucocephala x L. pulverulenta	Colombia
CIAT 22/9-7	L. pulverulenta x L. leucocephala	Colombia
CIAT 25/5 6	L. diversifolia	Colombia
CIAT 26/78 49	L. diversifolia	Colombia
CIAT 30/4-11	L. diversifolia	Colombia

Table [illegible] accessions

Access[illegible]	[illegible]	Solut[illegible] 1, ppm 0.5	1.0	2.0	4 0
			n n		
		Solution Ca, 5 ppm			
[illegible]	[illegible]	2.2 a	2.3 ab	2.5 a	2.1 ab
[illegible]	[illegible] a	1.6 a	1.4 bc	1.4 b	1.3 b
[illegible]	[illegible] ab	2.0 a	2.4 a	2.1 ab	2.4 a
[illegible]	[illegible] ab	2.0 a	1.7 abc	1.8 ab	1.6 b
[illegible]	1.6 ab	1.2 a	1.3 c	1.3 b	1.4 b
		Solution Ca, 10 ppm			
[illegible]	12.2 a	2.3 a	2.1 a	2.3 a	2.4 a
[illegible]	9.3 b	2.4 a	1.6 a	1.7 ab	1.6 ab
[illegible]	8.2 bc	2.5 a	2.2 a	2.4 a	2.3 a
[illegible]	7.7 bc	2.1 a	1.8 a	1.7 ab	1.7 ab
[illegible]	6.6 c	2.0 a	1.7 a	1.4 b	1.3 b
		Solution Ca, 20 ppm			
[illegible]	7.3 a	2.8 a	2.3 a	2.1 a	2.2 ab
[illegible]	6.4 ab	2.8 a	1.4 b	1.9 a	1.6 ab
[illegible]	5.9 abc	2.6 a	2.3 a	2.1 a	2.6 a
[illegible]	4.9 bc	2.3 a	1.8 ab	1.7 a	1.7 ab
[illegible]	4.1 c	2.0 a	1.4 b	1.3 z	1.3 b
		Solution Ca, 40 ppm			
[illegible]	9.6 a	3.4 a	2.8 a	2.9 a	2.3 a
[illegible]	7.6 b	2.9 a	2.9 a	2.0 b	1.5 b
[illegible]	7.3 bc	3.0 a	3.0 a	2.7 a	2.1 ab
[illegible]	6.4 c	2.4 a	2.4 a	1.8 b	1.7 ab
[illegible]	4.3 d	2.3 a	2.5 a	1.8 b	1.4 b

[illegible] the same column not followed by the same letter are [illegible] tly different at the 0.05 level.

[illegible] on the growth [illegible] constant level of Al have been [illegible] Robinson and Jackson, 1964, Clarkson and S[illegible], 1970). While the mechanism involved in [illegible] action between Al and Ca is not fully understood, [illegible] noted that Al toxicity can be overcome by [illegible] levels of Ca in solution. Quantitative differences [illegible] plant accessions in this study were most pronounced [illegible] the 5 and 10 ppm Ca level. This preliminary experiment established the Ca level used in the following two experiments.

For brevity, the 5 ppm Ca level data are discussed here, with only limited reference to the effect of higher levels of Ca on the growth of the seedlings. In general, accessions UF-1 and UF-2 appeared to be the most vigorous seedlings over all Ca concentrations where no Al was introduced into the solution, while UF 1 and VI-1 exhibited the best seedling growth in solutions containing Al (Table 6 3). Regardless of Ca level, the 0-5 ppm Al level severely decreased root growth and no significant differences among accessions were found.

The upper roots appear to be extremely sensitive to the Al in the solution. Stunting, thickening and loss of flexibility of the taproot were noted in all accessions. Occasional discoloration of the root was also observed.

experiment suggest

among these Leuca

ated to screen a bro

for tolerance to Al.

Experiment

A marked overall reduction in root growth was not

among all 100 Leucaena accessions in response to the four

levels of added Al, compared to their growth in the 0.0 ppm

Al treatment. No accession included in this study exhibited

high degree of Al tolerance. The adjusted root growth

lengths of the top 10% accessions at each level of Al are

reported in Table 6-4. PI 300011 and PI 443734 ranked in

the top 5% of accessions for root growth at the four levels

of Al, except for PI 300011 at the 4.0 ppm Al level, where

it ranked in the top 10%.

PI 244470, PI 282467, and PI 282473 also exhibited a

moderate degree of Al tolerance, ranking in the top 5% in

either of the Al levels.

Experiment 3

Adjusted root growth for the top ranked 50% of

accessions at each Al level is reported in Table 6-5. In

general, accessions which grew the longest roots in

solutions containing 0.0 ppm Al were also the same

accessions which ranked among the top 50% in root growth

over all levels of Al. This supports the importance of the

genetic

Table [illegible]. Adjusted r[illegible] [illegible] [illegible] [

Table [illegible] Adjusted root [illegible] accessions grown in [illegible]

Accession	Boron (ppm)					
	0.0	[illegible]	[illegible]	[illegible]	2.0	[illegible]
[illegible]	1.9 bc*	[illegible]	[illegible]	[illegible]	2.9 bc	[illegible]
[illegible]	3.3 abc	[illegible]	[illegible]	[illegible]	2.7 bc	[illegible]
[illegible]	6.6 a	[illegible]	[illegible]	2.9 ab	3.4 ab	3.2 a
[illegible]	[illegible]	[illegible]	3.3 a	3.8 ab	2.6 bc	2.7 ab
28186a	5.0 bc	[illegible]	2.5 abc	2.9 ab	2.7 bc	2.8 ab
28186b	[illegible]	[illegible]	3.2 ab	2.9 ab	2.4 c	2.2 b
28186c	4.2 c	[illegible]	2.9 ab	2.8 ab	2.8 bc	2.2 b
28269d	4.5 bc	[illegible]	3.0 ab	2.8 ab	2.5 c	2.6 ab
29234b	[illegible]	[illegible]	[illegible]	3.1 ab	2.3 c	2.6 ab
29536d	4.1 bcd	[illegible]	2.9 ab	3.0 ab	2.6 bc	2.9 ab
29536d	[illegible]	[illegible]	3.3 a	3.0 ab	3.0 abc	3.0 ab
308544	4.5 bc	[illegible]	2.5 abc	[illegible]	[illegible]	[illegible]
414771	6.0 ab	[illegible]	3.0 ab	3.3 a	3.7 a	2.6 ab
CIAT 17-5/ 2-37	4.9 bc	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CIAT 10-12/ 3-3	5.9 ab	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

*Means in the same column not followed by the same letter are significantly different at the 0.05 level.

rcome a suboptimal environment as
s lt of the earlier Conetainer® study in
that ranked highest in growth on acid soils
at near neutral pH.

rous accessions, PI 308544, CIAT 17-5/2 27,
, exhibited the reverse reaction, i.e ,
too 50% of the accesions in the 0.0 ppm Al
tanking among the bottom 50% at any level
of added Al

Tolerance to acidity or Al appears to be related to some extent to the genetic vigor of the plant, particularly at the seedling stage of development. When screening a large number of plant genotypes for a given characteristic, the plant breeder must impose a set level of selection pressure in choosing the most promising material. A 20% level of selection in this experiment identified accessions VI 1, UF-2, PI 295363, PI 295364, and PI 414771 as superior in adjusted root length at two or more levels of Al. PI 295364 and UF-2, in particular, ranked among the top 20% at and 3 levels of Al, respectively.

Summary and Conclusions

Genetic differences in Al sensitivity appear to exist among the Leucaena accessions evaluated in these experiments; however, little evidence of Al tolerance was found In Experiment 2, accesions PI 300011, PI 443734, PI 274470, PI 282467, and PI 282473 appeared to be the most

toleran[illegible]
as a muc[illegible] indicated [illegible]
accessio[illegible], [illegible]64, and [illegible]
might also offer a potential sour[illegible] tolerance.

In comparison with the use of co[illegible]ainers® and a [illegible]
medium for screening large numbers of [illegible] accession[illegible],
these short duration nutrient solution studies were
unsatisfactory. The soils study permitted a more realistic
approach to evaluating plant growth on acid soils, whose
disadvantage of the longer experimental time frame was
offset by the advantage of obtaining nodulation, shoot and
root growth data.

CHAPTER VII
GENERAL SUMMARY AND [illegible]

The results of these studies indicate that while Leucaena shows promise as both a forage and biomass crop in Florida, its adaptation should be limited to soils of a neutral to alkaline pH.

The nematological portion of this study demonstrated general resistance to infection by both M. incognita and M. javanica exists among the Leucaena spp. tested. Although previous experiments by Thrower (1958) and Hessey (1961) offer conflicting results, only a very low incidence of root galling was noted on Conetainer® grown plants of the accessions tested here. Field grown Leucaena accessions inoculated with M. incognita did not exhibit any root galling. In contrast, A. vaginalis was severely galled in both Conetainer® and field experiments. Leucaena is a high yielding, quality forage and biomass producer which has inherent tolerance to local races of root-knot nematode. These characteristics make it worthy of further efforts to adapt it to broader use.

In the second series of experiments, Leucaena spp. were found to nodulate on acid, flatwoods soil (pH 4.2) with indigenous cowpea Rhizobium spp. Fluorescent antibody identification showed cell surface antigens in common with

and [illegible]15 Rhizobium strains. [illegible] indigenous Rhizobium [illegible] of Indigofera sp., Tephrosia [illegible] were infective and effective on L. [illegible]ng fe?, but large, multilobed nodules. [illegible] these Rhizobium strains and TAL-1145 [illegible] of low pH, low P and high Al broth [illegible] period. Two isolates exhibited growth comparable to TAL 1145 in the treatment containing 50 ppm Al at pH 4.5.

In the third series of experiments, the variation for acid/Al tolerance among 98 Leucaena accessions was tested on three Florida soil types. Both field and Conetainer® studies confirmed that the plant has low inherent tolerance for acid/high Al conditions, exhibiting poor seedling development, stunting, chlorosis, and decreased nodulation. Significant differences among the accessions in plant [illegible], height, and nodulation were noted on all soil types; however, overall plant productivity on acid soils was poor.

In the final series of experiments, solutions containing Ca and varying levels of Al (0.0, 0.5, 1.0, 2.0, [illegible]) were investigated as a rapid method to evaluate [illegible]/Al tolerance among 100 Leucaena accessions. As a [illegible]g technique, use of the solutions proved to be more cumbersome and less informative than Conetainers® and soil. These results did support previous findings that significant

varia[illegible] pol[illegible] [illegible]nce accessions tested [illegible] the diff[illegible] in root growth were small in comparison with differences among the same accessions grown in solutions containing Ca alone.

In general, the results of these studies indicate that although Lencaena shows potential as a forage and/or biomass crop for Florida, its use may be somewhat limited by soil pH. It appears from these studies, that the plant is the limiting contributor in the symbiosis with Rhizobium spp. under adverse acid/high Al soil conditions. Future research should emphasize the adaptation and management aspects of the crop.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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